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EFFECT OF X-IRRADIATION OF RATS UPON THEIR RESISTANCE TO *TRYPANOSOMA LEWISI*

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Comparatively few studies of the effect of x-ray treatment of a host upon the course of a concomitant infection have been reported. Zinsser and Castaneda (1), by irradiating their animals, successfully increased the susceptibility of rats to typhus rickettsiae, and Liu, Snyder and Enders (2) irradiated mice with similar results. Kolmer, Rule and Werner (3) found that x-ray exposure, in the dosages used, did not render rabbits, guinea pigs, rats, nor ferrets susceptible to the virus of poliomyelitis. Corper (4) showed that thorium x had no effect on experimental guinea pig tuberculosis; Corper and Chovey (5) explain this finding on the basis that antibodies and circulating leucocytes are less important in a chronic infection like tuberculosis than in acute infections produced by pneumococci and hemolytic streptococci, which they showed to be enhanced by thorium x treatment of mice.

In the field of antibody production, Benjamin and Sluka (6) found that in animals irradiated before injection of beef serum the precipitin titer was lower and the antigen disappeared more slowly than in normal animals, but that irradiation four days after the injection of the antigen did not have these effects. Läwen (7) and von Heinrich (8) also found that irradiation does not affect antibody already formed and Hektoen (9) showed that production of lysin for red blood corpuscles was markedly reduced in animals which received large doses of x-ray at the start of immunization, although x-ray treatment given when antibody production was already well established had little or no effect upon the amount of hemolysin formed. He concluded that lymphoid tissue, which is the most susceptible to x-irradiation, is the source of antibodies.

Since, therefore, there is relatively little evidence on the influence of x-rays upon resistance, and that evidence conflicting, it was thought desirable to plan a series of experiments designed to study the effect of x-irradiation of a host upon the course of his infection and upon various aspects of his resistance. *Trypanosoma lewisi* was chosen as the infecting organism for this work because the course of infection with it in its natural mammalian host, the rat, so readily lends itself to quantitative determination. It was hoped that, if x-irradiation were found to influence the resistance of the host, some information might be gained both as to the reason for this effect and

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the normal defense mechanism of the rat to this parasite. While *Trypanosoma lewisi* is not a parasite of man, it is possible that knowledge gained from investigations into host reactions to it may also apply in human trypanosomiases.

MATERIALS AND METHODS

1. *The strain of rats.* All the rats used were of the Sherman strain and were maintained by the Department of Animal Care on a diet devised by Dr. C. A. Slanetz, consisting of:

Ground whole wheat	4050 g	$CaCO_3$	90 g
Commercial casein	900	Dried meat scraps (commercial)	600
Skim milk powder	600	Salt butter	450 (recently, butter 225 g, mazola 225 g)

All rats used in the experiments to be described were progeny of animals which had been rendered free of *Bartonella muris* infection by intramuscular injection with neosalvarsan, 0.015 g per 100 g of rat.[3] The rats were kept in a separate room. Their cages, food and bedding were sterilized before use. The cages were cleaned twice weekly and, in addition, fresh sterile cages were supplied once weekly. All rats were individually dusted at intervals of 3 to 4 weeks with "Pulvex," a commercial rotenone-containing powder, and the cage racks stood in cans holding kerosene. At the conclusion of each experiment, one animal of each series was splenectomized and held for three weeks. During this time, spreads of the tail blood were made at frequent intervals and carefully examined, after staining with Giemsa's stain, for evidence of *Bartonella muris*. At no time were any infected erythrocytes found.

2. *The strain of trypanosome.* The strain of *Trypanosoma lewisi* used in this work was kindly supplied by Mr. Henderson of the National Institute of Health. It was one which grew well at room temperature on slants of 10% rabbit blood-nutrient agar, overlaid with nutrient broth. Following intraperitoneal inoculation into normal rats, the prepatent period was 1 to 4 days and the peak of the infection was generally attained on the seventh to the tenth day. Trypanosomes used for infection were obtained either from a seed rat whose blood was diluted with nutrient broth, or from a young culture of *Trypanosoma lewisi*. The infecting doses ranged from 10,000 to 150,000 organisms, all the animals of each group receiving the same dose, as recorded in the protocols below.

3. *Procedure for irradiation.* X-ray exposures were very kindly performed by the late Dr. H. H. Kasabach and his assistants in the Department of Radiotherapy, Presbyterian Hospital. The rats were so placed in cardboard or wooden boxes that they could not climb over each other. The aperture of the target was large enough to cover the surface of the box, so that each rat was irradiated over its entire body. The following factors were used: 200 kv, 25 ma, a filter of 1 mm Al and inherent

[3] Because many of the rats maintained at this institution are infected with the common rat parasite, *Bartonella muris*, a preliminary study was made to determine whether the presence of this infection affected the resistance of rats to x-irradiation. Following irradiation, there was an immediate sharp decrease in circulating leucocytes and a delayed drop in erythrocytes, in all experimental and control rats. For the *Bartonella*-free rats, the average minimum white count was 552 per cu mm, reached in 2 to 4 days, and the minimum red count, 3.1 per cu mm, at 12 to 14 days; for the *Bartonella*-infected rats, the corresponding values were 370 and 2.4. For this reason and because survival time following x-ray tended to be less among the *Bartonella* carriers, it was decided to use only *Bartonella*-free rats for the work to be described.

filter of 0.15 mm Cu; 50 cm target-skin distance. The intensity of radiation was 125 to 140 r per minute, measured in air.[4] The dosage used in each experiment is stated below.

4. *Trypanosome counts.* (a) *Total counts.* Trypanosome counts on the tail blood of infected animals were carried out with a hemocytometer, using Hayem's solution as the diluent, or when the infection was slight, by counting the number of parasites seen in 50 high-power microscope fields (×450) of whole blood.

(b) *Percentage of dividing parasites.* Two hundred unselected parasites were counted on Giemsa- or Wright-stained blood films. Only those organisms in which a nucleus or kinetoplast was observed to be undergoing division were considered as dividing. A parasite which was apparently reproducing to form more than two new individuals was counted as one dividing organism.

5. *Preparation of vaccine.* Sixteen-day-old rats were intraperitoneally inoculated with 0.1 ml of a 3-day culture of *Trypanosoma lewisi.* Eight days later, when trypanosome counts ranged from 720,000 to 1,400,000 per cu mm of tail blood, the rats were bled from the carotid artery. The blood was thoroughly mixed with sodium citrate solution, diluted with physiological saline and slowly centrifuged to remove all the erythrocytes. Formalin was added to the supernatant trypanosome suspension to make a final concentration of 0.5% formalin (0.5 ml of 40% formaldehyde per 100 ml). For some of the work, part of this suspension was then diluted in 0.5% formalinized saline to give 4.3 million trypanosomes per ml. Since the presence of the formalin apparently rendered injections of this suspension very painful, the remainder of the suspension was centrifuged at high speed, and the organisms were washed and resuspended in saline containing 0.3% crude cresol. There were 4 million trypanosomes per ml in this phenolized vaccine. The sterility of the final suspensions was proved by their failure to cause infection in rats inoculated with them.

6. *Agglutinin titrations.* Rat sera were serially diluted in 0.9% saline solution. 0.15 ml of the vaccine described above (containing 4,000,000 trypanosomes per ml) was added to an equal amount of each serum dilution. After thorough mixing of antigen and serum, the tubes were placed in an incubator (37° C) for one hour and then in a refrigerator (10° C) for 20 to 24 hours. The tubes were examined in the light of a 40-watt bulb and the degree of agglutination estimated. Control tubes of serum 1:10 as well as of antigen suspension 1:2 were carried through the same incubation procedures.

EXPERIMENTAL WORK

PART I

Effect of X-ray upon Experimental Infections with Trypanosoma lewisi

Younger rats are known to suffer longer and more intense *Trypanosoma lewisi* infections than older rats (11, 12). For this reason and since the effect of irradiation

[4] In a preliminary experiment, it had been found that larger rats survived higher exposures for a longer time than smaller ones. Since larger animals get proportionally more irradiation because of more scatter and greater absorption, it was apparent that the older rats were capable of sustaining much larger amounts of x-ray than younger ones, and that absolute x-ray dosage could not be used in this work since the magnitude of the effect of irradiation would depend on the age of the recipient. It was found experimentally that the maximum x-ray dosage which uniformly permitted the survival in reasonably good nutrition of normal, uninfected rats was 300, 400, 500 and 500 r, respectively, for 15-, 25-, 35- and 60-day-old rats. It may be mentioned that these values, at least for the oldest group, are somewhat higher than those given by Leach and Sugiura (10) who reported that all 7 of their 2½- to 3½-month-old rats that were exposed to 500 r died within 18 days, some on the 2nd day.

TABLE 1.—*Effect of X-irradiation upon the Number of Circulating Trypanosomes*

No. of rats	Series	Age when infected (days)	X-ray (r)	No. of trypanosomes in infecting dose	Average parasite counts* on tail blood on designated days after infection															
					3	4	5	6	7	8	9	10	11	12	14	16	18	20	22	24	26	28
3	D	15	300	150	0	+	++	...	545	683	1200	...	875	597	562	339	373	353	400	303	277	356s
3	E	15	300	10	+	9	...	254	...	1533	1267	1650d	...	...	...	...	...	...	...	...	...	...
3	D	25	400	150	+	++	196	...	159	520	...	713	...	653	917	463	650	597	508	448	352	393s
3	E	25	400	10	+	143	...	1433	...	1315	...	1030	...	935‡	1150	1050	...	750	650	585	670	600s
3	D	35	500	150	+	+	12	...	67	570	...	893	...	950	690	600	815	533	625	605	320	445s
3	E	35	500	10	+	...	73	...	...	877	...	902	1047	1050†	...	680†	650	500	...	360	375	480s
2	D	60	500	150	+	...	30	138	...	370	...	425	...	665	595	783	680	455	283s	...	...	...
3	E	60	500	10	14	...	345	...	812	...	1092	...	758	718	...	519	513	490	364	377	302	350s
3	D	15	...	150	0	+	++	...	165	283	293	...	215	212	245	128	109	116	172	82	79	132s
3	E	15	...	10	0	+	...	75	...	468	560	667	...	367	...	408	241	117	47	109	51	54s
3	D	25	...	150	+	+	11	...	33	15	...	13	...	13	32	25	22	15	11	18	13	19s
3	E	25	...	10	+	37	...	233	...	376	...	195	...	122	126	150	90	78	81	77	70	35s
3	D	35	...	150	+	+	15	...	26	8	...	15	...	12	11	12	21	23	11	16	19	4s
3	E	35	...	10	0	...	35	...	...	69	...	150	64	91	...	43‖	...	38	118	70	59	45s
2	D	60	...	150	+	...	26	104	...	77	...	40	...	56	43	29	5	+	0s	...	...	...
3	E	60	...	10	10	...	117	...	194	...	173	...	157	109	...	70	102	94	89	101	64	45s

In each series, all the rats of the same age were litter-mates; Series D was started in July, 1942, E, in Sept.–Oct., 1942.

* 0 represents absence of trypanosomes in 50 microscope fields (×450).
\+ " less than one trypanosome per microscope field (×450).
++ " 1 to 10 trypanosomes per microscope field (×450).

The figures, 150, 10, 165, etc., represent thousands of trypanosomes/cu mm.

† One rat of group died.

‡ Two rats of group died.

‖ Infection cleared in 1 rat.

s indicates that rats were sacrificed; d indicates that rats died.

upon the course of the infection might manifest itself variously in animals of different ages, rats were selected from 4 age groups: nurslings (15 days), young rats just being weaned (25 days), adolescents (35 days), and adults (60 days).

Effect of x-irradiation upon the intensity of infection. The course of *Trypano-*

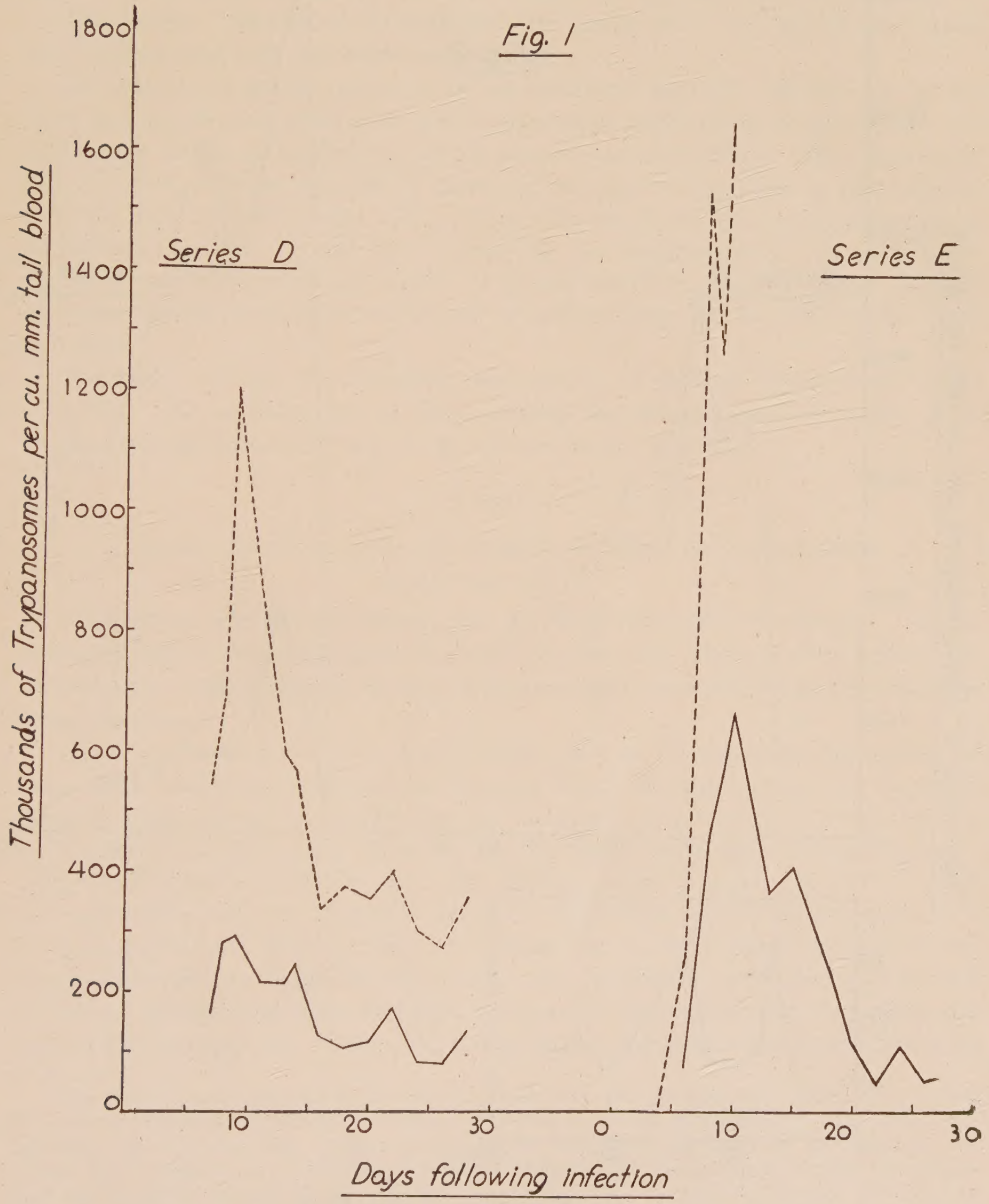


FIG. 1. 15-day-old rats, —— infected only; – – – – irradiated and infected.

soma lewisi infection in 46 rats, ranging in age from 15 to 60 days at the time of irradiation was followed. All the rats were infected within one hour of exposure of the test animals. The effect of the irradiation upon resistance, as revealed by the subsequent trypanosome counts, may be seen in Table 1 and Figs. 1–4.

The prepatent period varied from 1 to 4 days and appeared to be independent both of the size of the infecting dose and the factor of irradiation. The peak of the

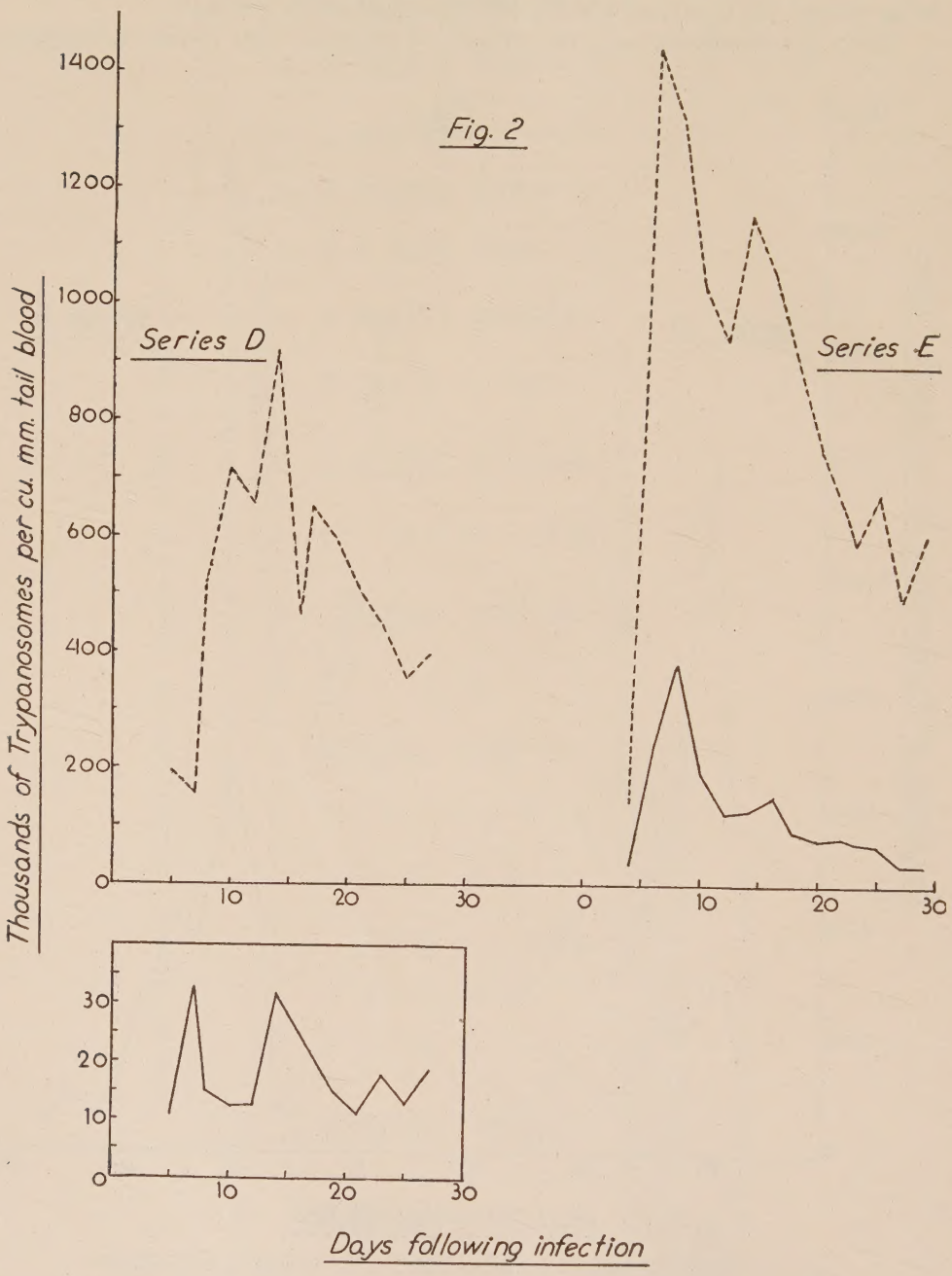


FIG. 2. 25-day-old rats, —— infected only; - - - - irradiated and infected.

infection was significantly higher in each of the irradiated groups than in their non-irradiated controls. Similarly, the decline in the number of circulating trypanosomes was slower in the irradiated groups. Because the strain of *Trypanosoma lewisi* used

tends to produce a prolonged chronic infection, the experiments were arbitrarily terminated at the end of 27 to 29 days, if the infection had not already disappeared by that time. Although it is therefore not possible to determine whether there was any difference in the duration of infection in the two groups, it is perhaps suggestive that the 3 rats whose infections did clear within 4 weeks were all unirradiated.[5]

Rat weight. This index of metabolism was compared in 3 groups of rats, irradiated, infected and both irradiated and infected.

Retardation of growth occurred in the irradiated animals. In each age group, those rats which were infected as well as irradiated made poorer progress than, and never quite caught up to, their irradiated, non-infected littermates. This observation indicates that whereas infection of otherwise normal rats has little if any influence upon the rate of development (15), infection in irradiated rats produces a definite inhibition of growth beyond that imposed by the radiation itself; i.e., irradiation appears to render the rats less capable of normal metabolic processes in the presence of *Trypanosoma lewisi* infection, as well as making them less resistant to the infection itself.

Mortality. Of the 23 irradiated infected rats, 8 died of *Trypanosoma lewisi* infection. The mortality rate in these animals was therefore approximately 35%, whereas among the control rats, none of which died, it was nil.

Part II

Factors Contributing to the Irradiation Effect on Trypanosoma lewisi *Infection*

Both cellular and humoral factors may play important roles in resistance. In the following experiments an attempt was made to determine which of these factors was so affected by x-ray treatment of the rats that increased susceptibility to *Trypanosoma lewisi* resulted.

Effect of x-irradiation upon the leucocyte level in Trypanosoma lewisi*-infected rats.* The established relationships between blood leucocyte levels and course of infection suggested the possibility that the increased susceptibility of irradiated rats to *Trypanosoma lewisi* might be correlated with the marked drop in circulating leucocytes which follows irradiation, although Culbertson and Kessler (16) who studied the relationship between leucocyte count and agglutinin production in response to *Trypanosoma lewisi* vaccine, found no evidence that an altered leucocyte picture was responsible either for resistance acquired through vaccination or for the much greater resistance exhibited by older rats after vaccination, in contrast with that artificially acquired by younger rats. Duca (11) found that the leucocyte count in *Trypanosoma*

[5] In view of such experiments as those of Patel (13) who showed that 6000 r is the lowest dose which affects the virulence of *Trypanosoma brucei*, and of Halberstaedter (14) whose experiments demonstrated that 12,000 r is required to inhibit the reproduction and therefore the infectivity of *Trypanosoma gambiense*, it appeared unlikely that trypanosomes injected into irradiated rats would be affected by the radiation energy remaining in the host tissues. Nevertheless it was thought advisable to test this possibility. Accordingly, 3 previously infected 35-day-old rats were irradiated when they showed an average of 12,000 parasites per cu mm of tail blood. By the 8th day following exposure, all had trypanosome counts of over 1 million per cu mm; one died on the 13th day and 2 on the 15th day after irradiation. In 3 litter-mates irradiated and infected simultaneously, the circulating parasites reached approximately the same number at the peak of the infection and 2 died, on the 13th and 15th days. Apparently, therefore, there is no significant irradiation effect on circulating parasites when their rat hosts are x-rayed.

lewisi-infected rats rose and fell parallel with the rise and fall of the number of circulating trypanosomes and the leucocyte response may probably justifiably be taken as an indication of the general cellular response to the infection.

In the irradiated infected rats used in these experiments, the white blood counts

Fig. 3

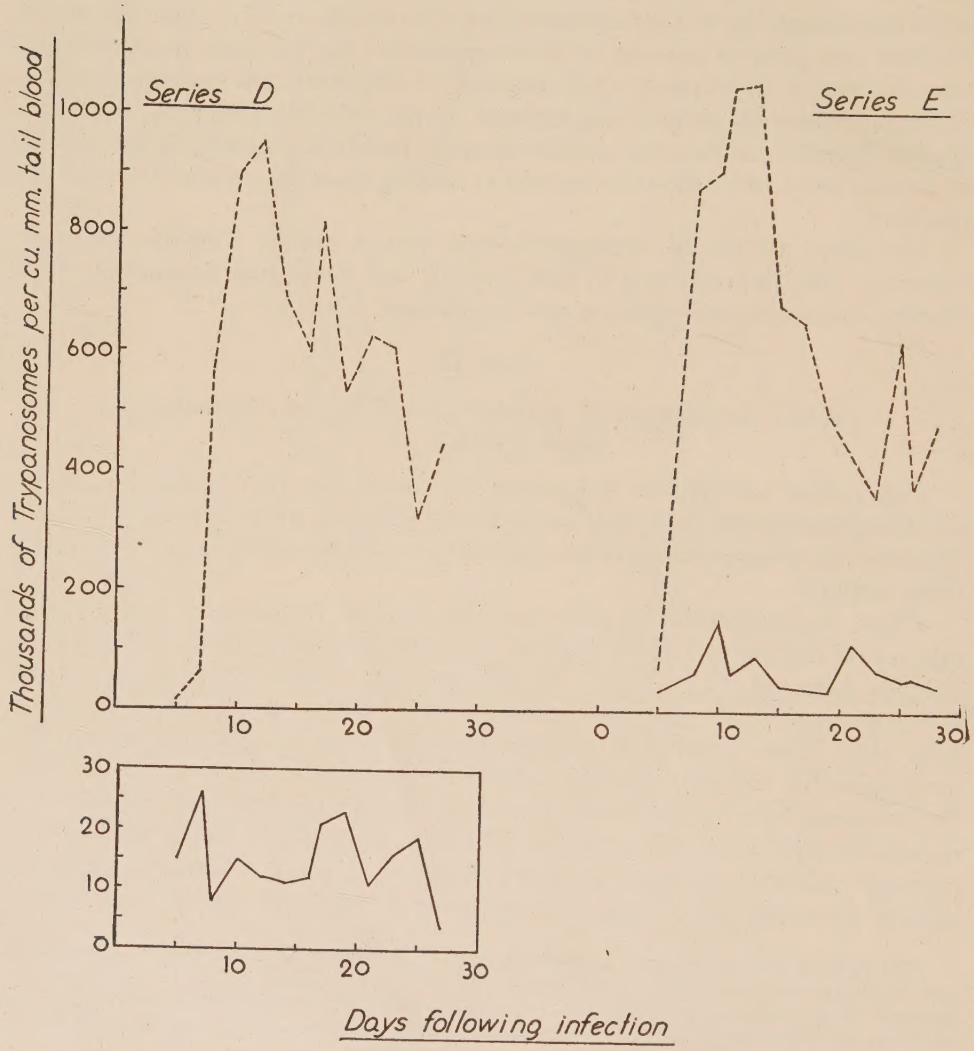


FIG. 3. 35-day-old rats, —— infected only; - - - - irradiated and infected.

reached a minimum, generally on the 4th or 5th day following irradiation, ranging from about 250 to 1500 leucocytes per cu mm. Shortly thereafter the counts started to rise. On the other hand, the unirradiated infected rats maintained counts of 15,000 to 25,000 per cu mm for the greater part of the acute infection. Table 2 shows

the time relationships between peak of infection, peak of white count and rise in white cells of irradiated animals.

The fact that the rise in circulating leucocytes in the irradiated rats started simultaneously with or before the peak of the infection or the peak white count occurred even in the normal rats probably indicates that the depressing effect of the x-rays upon resistance was not attributable to their influence upon the leucopoietic organs. In the rat, according to the observations of Latta and Ehlers (17), exposure to x-ray sufficient to depress the circulating leucocytes to 1000 per cu mm results in a reversal of the normal lymphocyte-granulocyte ratio from approximately 70% lymphocytes and 20% polymorphonuclear leucocytes to 20% and 70% respectively. In the present experiments, because of the extremely low white counts, running as low as 250 cells per cu mm, coupled with erythrocyte counts ranging to 8 or 9 million per cu mm during a good part of the infection, it was impossible to find sufficient leucocytes on blood smears to give accurate differential results. However, the indication was that the cells of the lymphocytic series were most severely affected by the irradiation. The

TABLE 2.—*Effect of x-irradiation upon the leucocyte count in* Trypanosoma lewisi-*infected rats*

No. of rats	Age when infected (days)	X-ray (r)*	Average number of days after infection		
			Rise in WBC began	Peak of WBC occurred	Peak of infection occurred
6	15	...	...	8.0	9.0
6	15	300	6.5	9.0	9.0
6	25	...	...	12.0	10.5
6	25	400	9.0	14.0	14.0
6	35	...	...	9.0	7.0
6	35	500	6.5	17.0	12.0
5	60	...	...	10.5	6.0
5	60	500	6.5	14.5	14.0

* X-ray exposure given one hour before infection.

rise in leucocytes which followed the initial drop was probably due to the regenerative powers of the spleen, lymph nodes and bone marrow, which were first demonstrated by Heinecke (18). A precipitous rise and fall in the leucocyte count of an occasional irradiated rat, long after the peak of the infection, was observed during the course of the present work. This may indicate that in some cases there was temporary overcompensation during the regeneration of the leucopoietic centers.

It has been shown above that the circulating leucocytes were probably not responsible for the effect of x-ray in diminishing resistance to *Trypanosoma lewisi.* Intense activity of reticular and endothelial cells in *Trypanosoma lewisi* infections has been demonstrated (19, 20) and it has frequently been shown (21, 22, 23, 24) that macrophage tissue is concerned in the formation of lysin and of ablastin antibody as it is known to be in the formation of other antibodies (25). Those very cells which appear to be responsible, in large measure at least, for resistance to *Trypanosoma lewisi* are also severely affected by irradiation (18, 26, 27). Their importance in resistance is probably not due solely to their phagocytic ability, as originally suggested by Laveran and Mesnil (28). It is still a moot question whether the prime factor is their ability to form a trypanolysin (29, 30), or ablastin in addition to a lysin (20, 31), or whether both factors are involved (22). In an attempt to explain the effect of irradiation on the course of *Trypanosoma lewisi* infection upon a humoral basis, several questions suggested themselves. Was the effect due to an inability (or

delayed ability) to produce antibody, or was it due to an inability to utilize antibody that was present?

Effect of x-irradiation of rats upon ablastin production. Taliaferro (20, 23, 32) and Coventry (31) have stated that in "non-pathogenic" trypanosome infections, the

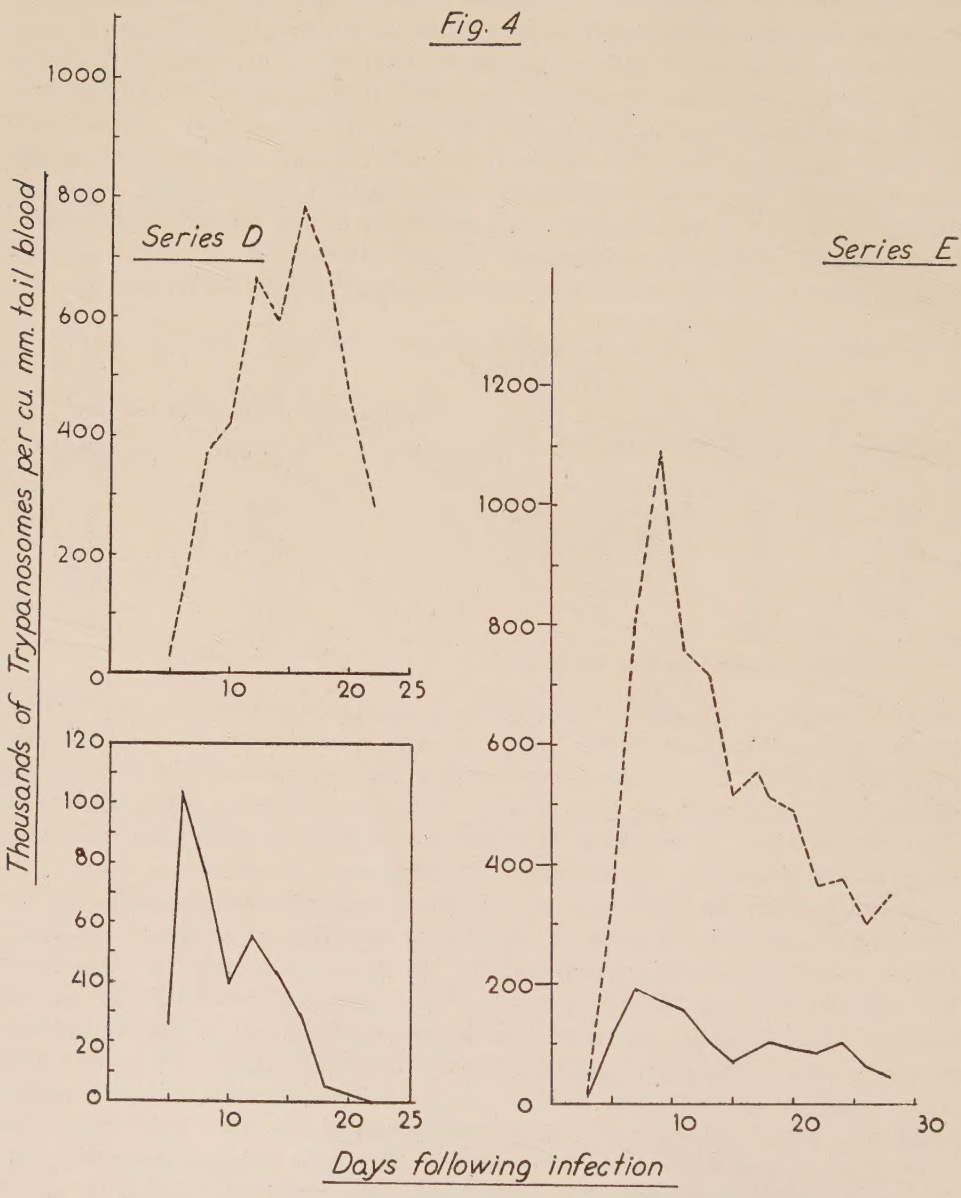


FIG. 4. 60-day-old rats, —— infected only; – – – – irradiated and infected.

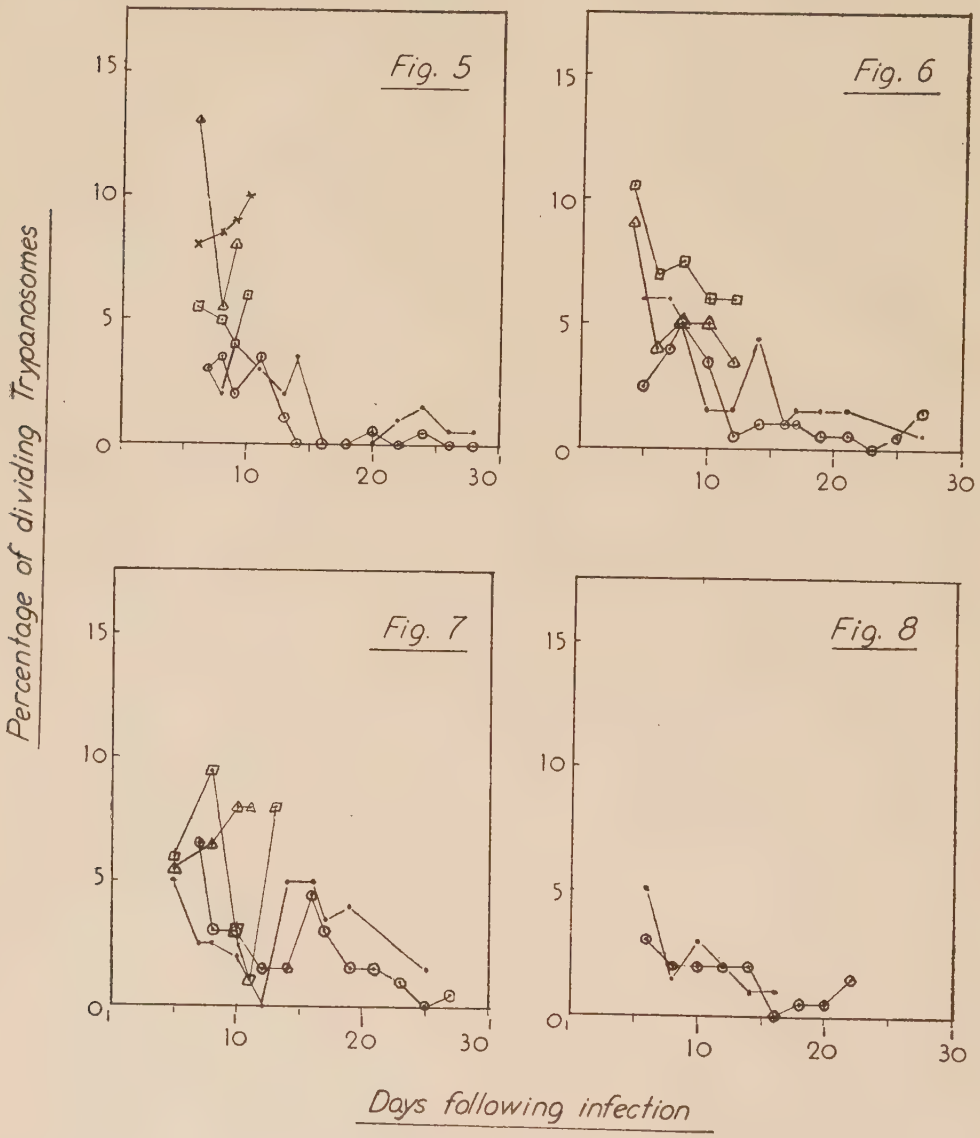
initial resisting mechanism is the production by the host of an ablastin antibody which completely inhibits reproduction. This is followed by the periodic production of a trypanolysin, so that the infection clears by a succession of number crises. Taliaferro

TABLE 3.—*Effect of x-irradiation of rats upon ablastin production*

Rat No.	Age when infected (days)	X-ray (r)	Total parasite counts per cu mm of tail blood and the percentage of dividing parasites on designated days following infection* †																	
			4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28
E4 ...	15	300	...	...	192 8.0	...	1600 8.5	1100 9.5	1200 10.0	d.	...	...	...	...	...	...	...	...	...	...
E5 ...	15	300	...	...	169 5.5	...	1400 5.0	1800 4.0	2100 6.0	d.	...	...	...	...	...	...	...	...	...	...
E6 ...	15	300	...	...	400 13.0	...	1600 5.5	900 8.0	d.	....	...	...	...	...	...	...	...	...	...	...
E25 ..	25	400	68 10.5	...	1450 7.0	...	1600 7.5	....	810 6.0	....	860 6.0	d.	...	...	...	...	...	...	...	...
E27 ..	25	400	260 9.0	...	1450 4.0	...	945 5.0	....	1180 5.0	....	886 3.5	d.	...	...	...	...	...	...	...	...
E13 ..	35	500	...	50 6.0	....	...	620 9.5	....	1300 3.0	940 1.0	...	400 8.0	d.	...	...	...	...	...	...	...
E15 ..	35	500	...	120 5.5	....	...	1200 6.5	....	640 8.0	1100 8.0	d.	...	...	...	...	...	...	...	...	...
D14 ..	15	300	...	...	....	590 3.0	700 3.5	1200 2.0	....	400 3.5	...	475 1.0	530 0.0	360 0.0	446 0.0	300 0.5	460 0.0	380 0.5	236 0.0	200 0.0
D9 ...	25	400	...	44 2.5	....	59 4.0	460 5.0	....	830 3.5	....	445 0.5	...	800 1.0	600 1.0	800 1.0	545 0.5	525 0.5	560 0.0	400 0.5	390 1.5
D2 ...	35	500	...	...	....	68 6.5	525 3.0	....	1050 3.0	....	700 1.5	...	680 1.5	760 4.5	900 3.0	440 1.5	610 1.5	380 1.0	140 0.0	310 0.5
D19 ..	60	500	...	...	36 3.0	...	460 2.0	....	340 2.0	....	630 2.0	...	650 2.0	710 0.0	710 0.5	540 0.5	215 1.5	s.	...	...
D16 ..	15	None	...	...	....	230 3.0	300 2.0	450 4.0	....	315 3.0	...	250 2.0	252 3.5	125 0.0	120 0.0	180 0.0	265 1.0	92 0.5	96 0.5	96 0.5
D11 ..	25	None	...	28 6.0	....	30 6.0	18 5.0	....	18 1.5	....	18 1.5	...	32 4.5	26 1.0	20 1.5	23 1.5	7 1.5	8 ...	10 ...	28 0.5
D5 ...	35	None	...	20 5.0	....	30 2.5	40 2.5	....	22 2.0	....	8 0.0	...	14 5.0	20 5.0	30 3.5	30 4.0	8 ...	25 ...	28 1.5	8 ...
D22 ..	60	None	...	...	96 5.0	...	80 1.5	....	240 3.0	....	233 2.0	...	25 1.0	15 1.0	8 ...	5 ...	Clear	...	...	...

* Upper figure in each row = parasite counts in thousands per cu mm of tail blood.
† Lower figure in each row = percentages of dividing forms in 200 parasites examined in stained blood films.
d. indicates that rat died.
s. indicates that rat was sacrificed.

(23) cites cases of apparently lethal infections with *Trypanosoma lewisi* in which reproduction of the parasites was never completely inhibited, and Culbertson and Wotton (33) showed that among a group of infected rats only those died which exhibited a high proportion of dividing parasites. The amount of ablastin present



FIGS. 5–8. Rate of reproduction of trypanosomes. FIG. 5. 15-day-old rats. FIG. 6. 25-day-old rats. FIG. 7. 35-day-old rats. FIG. 8. 60-day-old rats.

rats infected only.

rats irradiated and infected, which survived.

rats irradiated and infected, which died.

can be estimated by determining the proportion of parasites undergoing division. It is of interest to do this since a comparison of successive total parasite counts does not necessarily give an accurate picture of the course of the infection, for a gradual increase in the number of organisms may mean either that the parasites are reproducing slowly, or that a rapid rate of reproduction is almost paralleled by the rate of destruction. The greater intensity of infection noted in irradiated rats therefore reflects either their relative inability to inhibit reproduction, or a decreased ability to destroy the parasites, or both. Table 3 and Figs. 5 to 8 show the rate of reproduction of the trypanosomes infecting representative rats of each age group.

Among the rats which survived the infection, there seemed to be no significant difference between experimental and control animals in the percentage of dividing forms occurring at different stages of the infection. The fact that those rats which died (all young) permitted a high level of reproduction may mean that in them, the irradiation was effective enough to destroy even the ablastin, which younger rats normally produce in smaller amounts, whereas in those which survived, ablastin was successfully produced although lysin production was delayed or diminished. It is difficult to determine whether this indicates that the ablastin and lysin are different manifestations of the same antibody, the manufacture of the ablastic property being less injured by irradiation than that of the lytic, or whether there are two antibodies, produced by different cell types which in turn showed a differential sensitivity to x-ray. It was clear, however, that irradiation exerted a marked effect upon the lytic antibody (since a high level of infection persisted longer in the irradiated animals, even though the reproduction rate was no higher than in the controls) as well as upon the ablastic property of those rats which died.

Effect of x-irradiation upon antibody production in response to Trypanosoma lewisi *vaccine.* Of the 19 rats used in this experiment, 9 were irradiated and vaccinated and 7 were vaccinated but not irradiated. Three other rats served as additional controls, one being irradiated and the other two neither irradiated nor vaccinated. Six vaccine injections (4.0 or 4.3 million trypanosomes per 100 g rat) were given at 3-day intervals, the first coming on the day of irradiation. All the rats were infected with 130,000 to 150,000 trypanosomes on the 5th day following the last vaccine inoculation. Some of these animals were bled for serum samples just prior to the first vaccination and on the day of infection; all were bled at the end of the infection or at the conclusion of the experiment.

Of the 9 irradiated and vaccinated rats, 7 were successfully infected, whereas the parasite did not become established in any of the non-irradiated animals after immunization. It will be noted in Table 4 that the irradiated rats fell into two groups on the basis of their resistance following vaccination, one group exhibiting marked infection, the other showing either slight or no infection. Despite this difference in ability to develop immunity in response to vaccine, however, it was apparent that irradiation had the effect, in most instances, of sharply diminishing the ability to produce protective antibody. This finding was reinforced by the agglutinin titers of serums obtained five days after the last vaccine injection, shown in Table 5. In the control rats this was 1 : 320, while in the irradiated rats it was only 1 : 40, although two of the latter were nevertheless resistant to infection.

Effect of x-irradiation upon passive protection against Trypanosoma lewisi. These experiments were carried out to ascertain whether irradiation influenced the

TABLE 4.—*Effect of x-irradiation upon antibody production in response to* Trypanosoma lewisi *vaccine*

Rat No.	Age when vaccine series began (days)	X-ray (r)	Vaccine*	Parasite counts† on tail blood on designated days following infection‡													
				2	4	6	8	10	12	14	16	18	20	22	24	26	28
F1	31	400	+	0	0	++	290	363	d.								
F5	26	400	+	0	0	++	640	980	925	800	580	486	418	320	390	370	284
F7	26	400	+	0	+	++	230	211	179	112	100	114	86	112	128	62	98
J2	35	500	+	0	+	75	80	48	32	32	52	35	40	36	22	26	24
J4	35	500	+	0	+	88	153	90	68	76	58	60	60	36	72	77	61
F6	26	400	+	0	0	+	36	42	31	32	14	11	10	28	8	35	6
F8	26	400	+	0	0	+	4	25	21	+	0	0	0	0	0	0	0
J1	35	500	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J3	35	500	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F3	31	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F4	31	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F9	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F10 ...	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F11 ...	26	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J5	35	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J6	35	...	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
J7	35	...	.	0	+	49	36	31	18	10	0	s.	...	...	...	...	...
J8	35	...	.	0	0	+	23	80	78	44	52	11	40	36	27	10	0
J10 ...	35	500	.	+	35	132	412	230	154	83	92	72	108	100	21	0	0

* All the vaccinated rats received 6 intraperitoneal injections of 4.0 or 4.3 million trypanosomes per 100 g of rat, at 3-day intervals.
† Key to parasite counts: Same as Table 1.
‡ Infecting dose of 130,000 or 150,000 trypanosomes given 5 days after last vaccine injection.

TABLE 5.—*Effect of x-irradiation upon agglutinin titer following vaccination*

Rat No.	Age when vaccine series began (days)	X-ray (r)	No. of vaccine injections at 3-day intervals	Infecting dose given 5 days after last vaccination (thousands of trypanosomes)	Became infected	Highest dilution of serum (×2 after addition of antigen) in which agglutination occurred		
						Before vaccination	5 days after last vaccination	33 days after last vaccination
F5	26	400	6	150	Yes	....	.....	1:40
F6	26	400	6	150	Yes	....	.....	1:40
F7	26	400	6	150	Yes	....	.....	1:40
F8	26	400	6	150	Yes	....	.....	1:80
J2	35	500	6	150	Yes	1:40	1:40	1:160
J4	35	500	6	150	Yes	1:80	1:40	1:160
J1	35	500	6	150	No	1:20	1:40	1:40
J3	35	500	6	150	No	1:20	1:40	1:40
F4	31	...	6	150	No			1:320
J5	35	...	6	150	No	1:40	1:320	1:80
J6	35	...	6	150	No	1:20	1:320	1:80

efficacy of immune serum administered for protective purposes. Culbertson (34) showed that 0.25 ml of *Trypanosoma lewisi* antiserum per 10 g rat weight, given intraperitoneally on the day of infection, completely protected rats of various ages. Twelve of the 20 rats used for this experiment received intraperitoneal injections of pooled immune rat serum. Of these 12 rats, 8 had been irradiated within the previous hour. All, plus the controls, received the infecting dose of 100,000 trypanosomes subcutaneously to eliminate the possibility of immediate union of antibody and organisms in the peritoneal cavity.

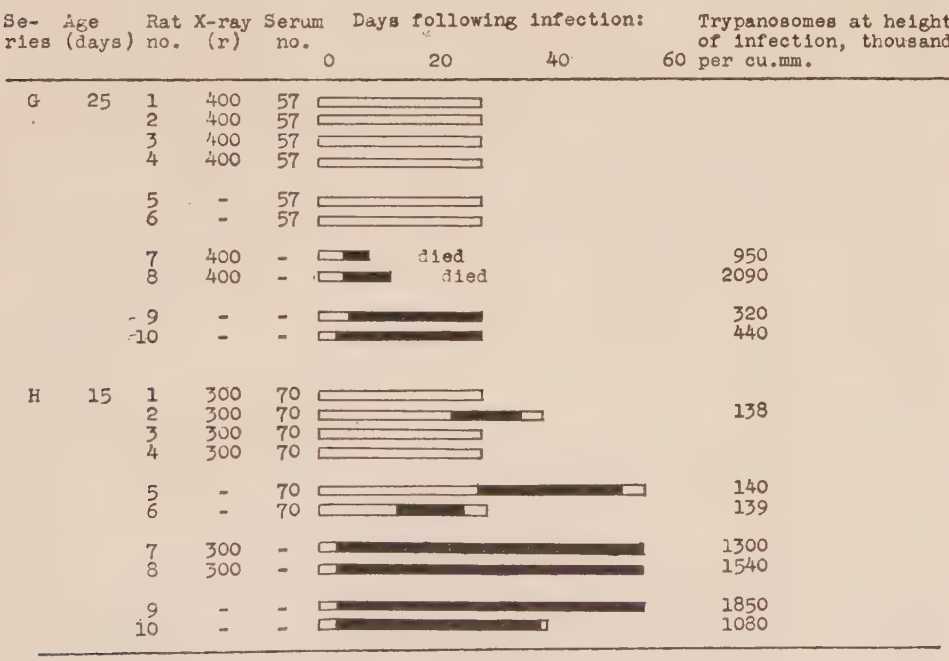


FIG. 9. Effect of x-irradiation upon passive protection against *Trypanosoma lewisi*. Length of rectangle indicates period of observation; blacked-in areas indicate duration of *Trypanosoma lewisi* infection. Serum 57: pooled from serum of 3 rats 25 days old at infection and 3 rats, 60 days, all nearly recovered at bleeding 4 weeks later; dose, 0.25 ml per 10 g rat. Serum 70: pooled from 3 rats 25 days old at infection, 2, 15 days and 2, 60 days, all either nearly or just recovered at bleeding 4 weeks later; dose 0.30 ml per 10 g rat to allow for previous dilution with merthiolate.

It was apparent (Fig. 9) that the 15-day-old rats of series H were less well protected by the serum given them than were the 25-day-old rats of series G. Whereas all of the latter were completely protected, 3 of the former became frankly infected, although only one of these had been irradiated. Even in these animals, however, the serum clearly had some effect, since in each case the prepatent period was prolonged, the duration of the infection markedly reduced, and the peak of the infection very much lower than in their litter-mate controls. Since even those rats which showed infection were partially protected, it may be assumed that both sera contained ablastic as well as lytic antibody. Taliaferro (35) has shown that serum containing ablastin is as effective in splenectomized blockaded rats as in normals. However, in normal animals, the passive ablastic immunity which lasts only a few days is replaced by a developing, active ablastic immunity whereas in the reticulo-endothelium-depleted animals, active ablastic immunity does not develop. This might explain the present finding that infection, where it did develop, was considerably delayed; that is, the infection developed after the disappearance of the passively introduced ablastin. Apparently most of these rats were able to manufacture ablastin and later lysin. Although some were not, this ability was not affected by the x-ray treatment.

Two factors may have been operative in the difference in protection afforded the two groups (G, H) of rats, namely the actual protective titer of the serum used and the age of the rats. Since the sources of the sera were approximately equivalent, there should not have been much difference between their protective values. To explain the observed differences in protective action on the basis of age differences between the two groups necessitates reconciling the discrepancy between the observations of Culbertson (34) that rats as young as 10, 15, and 20 days of age were completely protected by immune serum, and the present results. The assumption that his sera were more potent than those used here would resolve the difficulty, for it is well known that in immature animals, the macrophage tissue, of fundamental importance in the utilization of antibodies introduced into the body (35), is not fully developed functionally (36). Possibly the cooperation offered by immature macrophage tissue may not be sufficient, in the presence of a relatively weak antiserum, to resist infection completely.

For the purposes of this study, the important conclusion to be drawn from the data given in Fig. 9 is that irradiation did not significantly influence the effectiveness of antibodies introduced into the body simultaneously with the infecting dose. This may indicate, if true, that the effect of irradiation on the macrophage tissue is not exerted all at once but that before the effect is fully developed, the reticulo-endothelial system has already made its necessary contribution to the protective action of the serum.

Effect of x-irradiation upon latent Trypanosoma lewisi *infection.* Permanent resistance to reinfection by some protozoa (e.g., malarial plasmodia, *Endamoeba histolytica, Trypanosoma brucei*) has been shown to be of a latent infection (premunition) type and this may be true also in other cases in which proof of this type of immunity has not been obtained. Perla and Marmorston (36) state that since splenectomy does not stimulate recrudescence of the infection in a recovered rat, *Trypanosoma lewisi* infection is apparently not an example of a true latent infection. However, the essential mechanism of resistance to reinfection with *Trypanosoma lewisi* is still under discussion. It has been attributed to phagocytosis of the few

trypanosomes which may migrate into the blood stream from the site of inoculation (28), to ablastin plus phagocytosis (22) and to lysin plus ablastin (29, 30, 31). In recent work, Augustine (37) has shown that trypanosomes (*Trypanosoma lewisi*) introduced into the peritoneal cavity passed with equal ease through the lymph nodes and into the blood stream of normal and immune rats. He further showed (38) that immunity to reinfection with *Trypanosoma lewisi* seems not to be due to the presence of ablastin since, with a massive infecting dose, the trypanosomes which remained in the blood stream for 2 days could be seen to divide. Apparently the failure of the parasites to increase in number was caused by the opsonic or agglutinative effect of the parasiticidal antibody.

It was felt that the x-ray technique used in this work might serve to demonstrate whether latent infection does in fact occur with *Trypanosoma lewisi*. Accordingly, of 7 litter-mate rats, infected when very young, 5 were irradiated as soon as their blood stream infection had cleared. Frequent subsequent blood examinations showed no trypanosomes, except in the case of one irradiated rat. Although examination of this rat revealed a single circulating parasite on the sixth and seventh days following irradiation, it was apparently suffering an atypical infection because weeks later, when no parasites could be seen microscopically, its blood was still able to infect clean rats although the blood of an irradiated litter-mate could not. From these experiments, one can conclude only that if there is a latent stage of *Trypanosoma lewisi* infection, x-ray did not inhibit the defense mechanisms to the point where the organisms were driven into the blood stream.

DISCUSSION

The present study has shown that x-irradiation depresses the resistance of rats to *Trypanosoma lewisi* infection. The effect of the rays was to increase the intensity of the infection and to prolong the period of high trypanosome levels in the blood. The striking effect of irradiation upon the leucocytes, especially of the lymphocytic series, was apparently unrelated to its effect upon resistance. The fact that there is marked activity of reticulo-endothelial cells in *Trypanosoma lewisi* infections, coupled with the knowledge that this tissue is severely affected by x-ray, suggested the possibility that the inhibiting effect of irradiation upon resistance might be due to its interference with humoral mechanisms. In pursuing this hypothesis it was found that while there was no significant difference in the protective action of immune serum administered to normal and irradiated rats, the ability of the rats to produce antibody, both ablastic and lytic, was markedly diminished by irradiation. X-ray treatment inhibited antibody production not only in the presence of infection, but also in response to a series of vaccine injections. From this it appeared that the x-rays, known to destroy macrophage tissue, diminished resistance to *Trypanosoma lewisi* by decreasing the amount of antibody produced, since it is fairly well established that the initial defense mechanisms in this infection are humoral in nature. It may be concluded that macrophage tissue, particularly in its antibody-producing capacity, is of paramount importance in the resistance of rats to this infection.

Infection in irradiated animals tended definitely to inhibit their growth, indicating that irradiation not only made the rats more susceptible to infection but also rendered them less capable of normal metabolic processes in the presence of *Trypanosoma lewisi*. There may well be a direct relationship between the deficient metabolism of the animal as a whole and its increased susceptibility to infection.

The experimental results obtained by x-irradiation of host animals suggest that this technique might profitably be employed in studies where a partial and temporary elimination of the reticulo-endothelial system is advantageous. Provided the dosage is not excessive, regeneration of the injured tissue, especially in the bone marrow and lymph nodes, is quite complete (27).

SUMMARY AND CONCLUSIONS

X-irradiation of rats increased the severity of their *Trypanosoma lewisi* infection. In 46 rats of four age groups, 15, 25, 35 and 60 days old, respectively, at the start of the experiment, the peak of the infection ranged from 2 to 8 or more times as high in the irradiated animals as in the controls.

The ability of rats to produce ablastic antibody against *Trypanosoma lewisi* was markedly diminished by x-ray treatment. In those irradiated rats which failed to survive the infection, the proportion of reproducing parasites remained at approximately 5 to 8%, whereas in the survivors, both irradiated and control, the proportion declined in about 10 days to 0 to 2% from an initial high of 4 to 6%. Trypanolysin production also seemed to be affected by irradiation as indicated by the greater duration of a high level of infection even in those survivors whose ablastic level was diminishing.

Vaccination with a formalinized trypanosome suspension completely protected 100% of 7 normal rats from a subsequent infecting dose of 130,000 trypanosomes, whereas only 22% of 9 previously irradiated rats were similarly protected.

Previous x-irradiation of rats did not significantly influence the effectiveness of protective antibodies introduced simultaneously with the infecting dose of *Trypanosoma lewisi*.

The sharp reduction in circulating leucocytes which followed x-ray treatment appeared not to be a factor in the decreased resistance of irradiated animals, since the leucocyte level had returned to approximately the normal value at the time of the peak of infection.

Irradiation of rats recovered from *Trypanosoma lewisi* infection did not produce evidence of a latent stage of the infection.

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STUDIES ON THE VITAMIN REQUIREMENT OF TAPEWORMS[1]

C. J. Addis and Asa C. Chandler

The vitamin sources and requirements of tapeworms present an interesting problem not only from the parasitological point of view but also from the standpoint of the physiology of the host. The possible absorption of vitamins from the mucosa of the intestine may account for some of the pathogenic effects of parasitism by tapeworms, and there is an additional point of interest in an apparent relation between the vitamins and the sex hormones, as pointed out by the work of Chandler (1943).

Very little work has been done on the vitamin requirement of the cestodes. Hager (1941) found that there was a marked decrease in the egg production of *Hymenolepis diminuta* in rats when the hosts were kept on a milk diet and when soybean meal was substituted for yeast. A diet deficient in both vitamin B_1 and the G complex caused a very marked decrease in the egg output, with loss of the worms in some cases. There was no significant decrease in the egg output when only B_1 was eliminated from the diet, but the lack of the G complex alone did cause a decrease. Hager suggested that there is a factor in yeast associated with the G complex which is necessary for normal egg production by the rat tapeworm, but this factor is not necessarily a member of the G complex.

Chandler (1943), working with *Hymenolepis diminuta* in rats, studied the effect of variations in the diet of the host on the worm as determined by counting and measuring the worms after autopsy. He found that this worm is totally independent of protein in the diet of the host and drew the conclusion that the worms absorbed the nitrogenous substances directly from the mucous membrane. He suggested that this dependence of the tapeworm on the mucosa of the host for nitrogenous materials might explain the cause of the stunting effect of crowding, which has been repeatedly reported. He also found that the lack of vitamins in the diet, with or without the provision of proteins, had a marked effect on the establishment of the worms in female rats, while in male rats there was no apparent effect in either case. Also, there was a marked stunting in the growth of the worms in female rats on a vitamin-deficient diet with provision of proteins, but in female rats which lacked both vitamins and proteins the worms grew to the normal size. In male rats on a vitamin-deficient diet, with or without the provision of proteins, there was no effect on the growth of the worms.

In further experiments, Chandler showed that this worm is dependent on some factor contained in autoclaved yeast in the diet of the host, but the lack of the fat-soluble vitamins A, D, and E and vitamin B_1 had no effect on the growth of the worms in female rats.

Chandler suggested that there must be some intricate involvement of the female sex hormones with protein metabolism and vitamin utilization, which as yet has not been explained. He also expressed the view that tapeworm toxicity may be due to the absorption of proteins and vitamins, and possibly hormones or other substances, from the mucosa of the intestine of the host by the worms, instead of the absorption of toxic products of the worms by the host.

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The present investigation was carried out as a continuation of the work of Chandler (1943) to determine more exactly the relationship between the vitamin content of the diet of the host and the establishment and growth of the worms. The method used was similar to that of Chandler (1943). The rat tapeworm, *Hymenolepis diminuta,* was used in all the experiments and the hosts were laboratory-bred albino rats. The results were based on the number and size of the worms after autopsy.

The grain beetle, *Tenebrio molitor,* was used as the source of the cysticercoids. The beetles were infected by mixing the eggs obtained from a stock supply of infected rats with shaved apple and feeding it to the beetles. After 19 to 21 days the beetles were teased apart and cysticercoids were removed and washed. Each rat was then given 10 mature cysticercoids by means of a pipette.. In the following experiments only female rats were used, since Chandler (1943) showed that there was no effect of a vitamin-deficient diet on the worms in male rats. The rats were between 3 and 4 months old and weighed between 150 and 200 grams at the beginning of the experiments. Autopsies were run on the rats 14 days after being infected since at this time no segments have yet been shed, although the worms have as great an average length as they do if removed from the host at later periods. The worms were washed out of the intestines with warm water and were allowed to relax in cold water for two to three hours, after which they were counted and measured. All worms measured possessed a terminal segment and a scolex.

In the following experiments the rats were placed on diets varying in their vitamin content. The basic portion of the diet for six rats per day was as follows:

Fisher Ruffex[2]	10 gm
Casein, vitamin-test	18 gm
Dextrose	25 gm
Corn starch	25 gm
Corn oil	10 gm
Salt mixture	4 gm

For a complete diet for six rats per day, 9 gm of Harris' or Fleischmann's brewer's yeast were added to supply the B complex, and 3 ml of a mixture of 30 ml of cod-liver oil, 25 ml of wheat-germ oil, and 4 ml of halibut-liver oil to provide the fat-soluble vitamins A, D, and E. For a diet containing no B_1, 9 gm of autoclaved yeast was used. For a diet containing vitamin B_1 alone, a 1-mgm tablet of thiamin chloride was dissolved in 10 ml of 20 per cent alcohol and added to the basic portion for six rats per day.

The basic portion of the diet was mixed in such quantity as usually to last the length of the experiment. Each day the proper amount of this portion was weighed and the vitamin content added. On days when the entire amount of food given the rats the day before was not eaten, enough of the basic portion was added to bring it back to its normal weight, but the full amount of the vitamins was added to insure adequate amounts of them.

EXPERIMENT 1

In the first experiment four groups of six female rats were used. Each rat was weighed, fed 10 cysticercoids, and started on its experimental diet the same day,

[2] The Fisher Ruffex is composed mainly of alpha cellulose and was used to provide bulk; its food value is practically nil and all the vitamins have been destroyed or removed.

allowing no time for the depletion of the host's vitamin store. Group I was given a complete diet; group II was given a diet which lacked all the vitamins; group III was given a vitamin B_1- and G-complex-deficient diet; and group IV was put on a diet deficient in vitamins A, D, E, and the G complex. After 14 days the rats were autopsied and a comparison of the number and size of the worms was made.

The results of this experiment, as shown in Table 1, show that the lack of vitamins in the diet of the host has some effect on the establishment and growth of the tapeworms even when no time is allowed for the depletion of the host. In group II, where there was a complete lack of all the vitamins, the number of worms that were able to establish themselves was somewhat less than that of the worms in the rats on the other diets. There was also a stunting in the growth of these worms. In group III, rats on a vitamin B_1- and G-complex-deficient diet, the average number of worms was the same as that in the rats on a complete diet, but the growth of the worms was stunted as much as it was in the vitamin-deficient rats. In group IV,

TABLE 1.—*No preliminary depletion*

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete)	+2.6	8.2	251.6–358.3	321.4	± 8.13	2,635.5	1.56
II (no vitamins) ...	−8.0	4.7	118.3–290.0	231.6	± 20.87	1,088.5	1.11
III (no B_1 or G complex) ...	−6.1	8.2	147.5–302.5	231.0	± 15.43	1,894.2	1.08
IV (no A, D, E, or G complex) ..	−6.1	6.0	183.6–295.0	247.1	± 13.34	1,482.6	1.15

* Ratios of $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$:
1. I to II = 4.03. 2. I to III = 5.17. 3. I to IV = 4.79.

no vitamins A, D, E, or G complex, the number of worms was lower than that in groups I and III but not as low as in group II. The average length of the worms was about the same as that of the worms in the rats with no vitamins and in those lacking vitamin B_1 and the G complex.

Although this experiment showed that the lack of vitamins in the diet of the host increases resistance to the establishment of the worms and causes a stunting in the growth of the worms that become established, the effect on the worms was not as marked as might have been expected. Consequently a second experiment was planned in which the host was put on a depletion diet for a certain length of time.

EXPERIMENT 2

In this experiment a depletion period of 4 days was allowed before the cysticercoids were fed. The four diets used in experiment 1 were repeated, and four additional groups were put on diets containing the remaining combinations of the three groups of vitamins. Group V was put on a diet containing none of the vitamin G complex; group VI, no vitamin B_1; group VII, no vitamins A, D, or E; and group VIII, no vitamins A, D, E, or B_1.

A study of Table 2 will emphasize the conclusions of experiment 1. The number of worms and their size in the vitamin-deficient rats were reduced still further. In the four groups containing none of the G complex, i.e., groups II, III, IV, and V, there was a loss of weight in the rats, as might be expected from such diets, and a

definite stunting in the growth of the worms. It can be seen that in group V, lacking the G complex only, and in group II, lacking all the vitamins, there was produced the greatest stunting in growth, while in groups III and IV, lacking vitamins B_1 and vitamins A, D, and E, respectively, as well as the G complex, the reduction in the length of the worms was not as great. Although the difference between the four groups is not statistically significant, as seen from the ratios, there is a suggestion that the absence of vitamins A, D, E, and B_1 may be favorable to the growth of the worms. The possible effect of these vitamins will be discussed further below. In the three remaining groups, VI, VII, and VIII, containing the G complex, the growth of the worms approximated that of the worms in the rats on a complete diet.

It can be seen that the lack of all the vitamins definitely decreases the number of worms that succeed in establishing themselves, but just what vitamin or group of vitamins is concerned is not very clear. Judging by the results in groups V and VII, it seems to be the fat-soluble vitamins, A, D, and E and the G complex that are involved, but in groups III and VIII, which lacked the G complex and the fat-soluble

TABLE 2.—*4 days depletion*

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete) ..	+16.5	7.8	220.9–376.8	326.6	±15.62	2,545.8	1.59
II (no vitamins)	−19.4	3.5	69.0–198.5	126.0	±21.43	491.4	0.78
III (no B_1 or G complex) ..	−11.1	6.2	150.0–239.2	210.0	±14.78	1,302.0	0.99
IV (no A, D, E, or G complex)	−13.1	5.0	80.0–215.8	171.9	±14.97	859.5	0.98
V (no G complex)	−10.2	5.5	126.3–195.0	140.3	± 9.82	771.7	0.82
VI (no B_1)	+10.5	6.4	195.0–266.3	255.3	± 9.12	1,663.9	1.37
VII (no A, D, or E)	+ 8.9	4.8	258.0–379.8	324.1	±16.68	1,555.7	1.46
VIII (no A, D, E, or B_1)	+10.9	6.8	308.9–344.8	318.9	±13.44	2,168.5	1.42

* Ratios of $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$:

1. I to II = 8.31.
2. I to III = 6.29.
3. I to IV = 7.06.
4. I to V = 10.11.
5. I to VI = 3.93.
6. I to VII = 0.10.
7. I to VIII = 0.36.
8. II to III = 3.23.
9. II to IV = 1.76.
10. II to V = 0.61.
11. III to IV = 1.82.
12. III to V = 3.94.
13. IV to V = 1.77.
14. VI to VII = 3.62.
15. VI to VIII = 3.93.
16. VII to VIII = 0.24.

vitamins, respectively, and also vitamin B_1, the number of worms present was close to that of the controls, and in group VI, lacking vitamin B_1 only, there was likewise no significant reduction in the number of worms present. It would seem from these data that the lack of either vitamins A, D, and E or the G complex inhibits the establishment of the worms, while there is a suggestion that the absence of vitamin B_1 is favorable to their establishment.

Because of the questions which arose from this experiment and in order to try to make the picture clearer, another experiment was planned in which the host was depleted of its vitamins for a longer time.

EXPERIMENT 3

In this experiment the same diets used in experiment 2 were repeated, allowing 17 days for the depletion of the host before feeding the cysticercoids.

A study of Table 3 shows that a longer depletion period in the host intensifies the effects of the lack of certain vitamins in the diet. In the rats on a diet deficient in the G complex there was a marked stunting in the growth of the worms, while in

the three groups which received an adequate amount of the G complex the worms reached a greater length than those in the controls. These results corroborate the need of the vitamin G complex in the diet of the host for the normal growth of the tapeworm, and provide further evidence that the worms grow larger, rather than smaller, in the absence of the fat-soluble vitamins and of vitamin B_1.

In this experiment, the decrease in the number of worms established in the absence of the fat-soluble vitamins is more marked than in experiments with shorter depletion periods. The absence of the G complex also seems to have some effect on the establishment but is not as pronounced as the lack of the fat soluble vitamins. The absence of the G complex along with that of vitamins A, D, and E intensifies the reduction in the number of worms established. In diet VI, where the rats were on a vitamin-B_1-deficient diet, there is no significant effect on the number of worms present.

TABLE 3.—*17 days depletion*

Diet	Change in wt. of rats (per cent)	Av. no. of worms at autopsy	Length of worms (mm)				Av. width of worms (mm)
			Range of av. in indiv. rats	Av. per worm*	Probable error of av.	Total per rat	
I (complete) .	+17.3	8.0	304.0–356.7	328.3	±16.48	2,626.4	1.58
II (no vitamins)	−18.6	3.2	56.3–133.0	101.1	± 8.58	323.5	0.69
III (no B_1 or G complex) ..	−13.2	5.7	107.4–249.0	190.9	±16.54	1,088.1	0.97
IV (no A, D, E, or G complex)	−15.1	3.0	141.7–197.0	166.4	± 9.58	499.2	0.96
V (no G complex)	−15.5	5.0	82.0–184.6	128.9	±11.13	944.5	0.68
VI (no B_1)	+11.1	6.7	328.4–424.9	339.4	± 6.42	2,274.0	1.57
VII (no A, D, or E).....	+10.2	3.8	305.0–572.4	388.5	± 6.58	1,476.3	1.61
VIII (no A, D, E, or B_1)	+ 9.6	5.3	366.5–480.2	399.4	± 5.73	2,116.8	1.62

* Ratios of $\frac{\text{Difference of averages}}{\text{Probable error of difference}}$:

1. I to II = 12.21.
2. I to III = 5.90.
3. I to IV = 8.48.
4. I to V = 10.02.
5. I to VI = 0.63.
6. I to VII = 3.38.
7. I to VIII = 4.09.
8. II to III = 4.83.
9. II to IV = 5.10.
10. II to V = 1.95.
11. III to IV = 1.28.
12. III to V = 3.12.
13. IV to V = 2.55.
14. VI to VII = 5.34.
15. VI to VIII = 6.98.
16. VII to VIII = 1.25.

DISCUSSION

The experiments described above indicate clearly that the rat tapeworm, *Hymenolepis diminuta,* is dependent upon the G complex in the diet of its host for normal growth, as previously suggested by the work of Hager (1941) and Chandler (1943); the stunting effect on the growth of the worms becoming progressively more marked with an increase in the length of the depletion period prior to the infection of the host.

It is probable that tapeworms require the same vitamins required by other organisms; they may acquire them: (1) from the unassimilated food of the host, (2) by absorption from the mucous membranes of the host itself, or (3) by synthesis. The first source has definitely been eliminated by the work of Chandler (1943) and by the present investigation for all except the G complex. Absorption from the mucous membranes, or synthesis, seems to afford an adequate source for all except the G complex or some part of it, since diets deficient in all but the G complex have no harmful effect on the growth of the worms. Since the effect of the absence of the G complex in the food of the host became progressively more marked as the host's depletion period was increased prior to infection, it is a logical

deduction that the missing vitamin or vitamins are not synthesized by the worms but are obtained by absorption from the host's mucous membrane. Only in rats well supplied with the G complex in the daily food is an adequate amount available, but even after long depletion enough can be absorbed by the worms to allow them to grow at a slower rate. It seems logical from this that an infection of tapeworms in a host living on a diet deficient in this group of vitamins would intensify the avitaminosis. Experiments are in progress now to determine the component or components of the G complex that are necessary for the normal growth of the worms.

Since the lack of the fat-soluble vitamins and B_1 in the diet of the host had no stunting effect on the growth of the worms after a long depletion period, and even produced an increase in the size of the worms over that of the controls, the possibility suggests itself that the worms may be able to synthesize any of these vitamins which they need for normal maintenance of growth and health. However, yeast autoclaved for an hour is not entirely depleted of thiamin, and "vitamin-test" casein contains traces of it. In a personal communication Dr. R. J. Williams informs us that samples of such casein tested in his laboratory contained from 0.13 to 0.28 micrograms of thiamin per gram, about one-tenth to one-twentieth of that found in a good food mixture. The worms could, therefore, obtain a small amount of thiamin from this source. Also, as pointed out by Dr. Williams, the tissues of an animal fed on a thiamin-deficient diet do not immediately fall to a low level of thiamin content, so the worms could possibly have derived enough of this vitamin from the mucous membrane of the host even after several weeks of depletion.

The increase in size of the worms in rats on diets deficient in the fat-soluble vitamins is in accordance with observations made by Ackert, McIlvaine, and Crawford (1931) on the nematode, *Ascaridia lineata,* in chickens on a vitamin-A-deficient diet. They attributed this increase in size to the partial paralysis of the intestine, thus producing a richer intestinal flora in the vitamin-A-deficient chickens than in the controls. In the present investigation it was noted at autopsy that the intestines of the rats deficient in the fat-soluble vitamins or vitamin B_1 were more compact and contained more fecal material than did the intestines of the other rats, indicating weakened peristalsis. This observation is in accordance with that of Ackert, McIlvaine, and Crawford (1931) and the several reports that vitamin B_1 is corrective of certain types of constipation. It seems probable that the increase in the growth of the worms in these cases, if not entirely accounted for by the smaller number of worms present, might be due to the weakened peristalsis. This partial paralysis of the intestine would allow the worms to become established in a more anterior position which, by analogy with the result obtained by Burlingame and Chandler (1941), would be more favorable for growth. No data were obtained on the position of the worms in the rats, so this conclusion is only tentative. Weakened peristalsis and possible establishment of the worms in a more favorable anterior position might also explain the moderate increase in size of the worms in the rats on a diet deficient in vitamins A, D, E, and the G complex (groups IV) and on a diet deficient in vitamin B_1 and the G complex (groups III) as compared with the worms in the rats on a diet deficient in the G complex alone (groups V). A study of the size of the worms in the individual rats does not warrant the conclusion that the increase in size is due to a smaller number of worms present. There is little correlation between the number of worms and the average length of the worms in the individual

rats of a dietary group. For example in groups III and IV in experiment 2 the number of worms and average lengths are as follows:

Group III			Group IV		
No. of rat	*No. of worms*	*Average Length*	*No. of rat*	*No. of worms*	*Average Length*
1	9	117.4	1	7	157.0
2	7	239.2	2	7	137.0
3	6	218.3	3	6	215.8
4	6	150.0	4	4	171.0
5	1	85.0	5	3	150.0
6	0	0.0	6	3	80.0

Apparently the number of worms present, up to 9 or 10 worms, has little influence on the length to which the worms grow during the first 14 days. The increase in length with diminution in numbers reported by Chandler (1939) is apparently due mainly to growth after the first 14 days.

The establishment of tapeworms would seem to depend upon the proper evagination of the scolices and their attachment upon the mucous membrane of the wall of the intestine. Exactly what causes the evagination in the host is not known, but it is probably dependent upon the digestive juices or the pH of the gut, and any disturbances along this line would affect the establishment of the worms.

The experiments above show that the lack of vitamins A, D, and E decreases the number of worms established. This diminution in the number of worms present in rats on a diet deficient in the fat-soluble vitamins is in contrast to the results of Ackert, McIlvaine, and Crawford (1931), who found that the resistance of chickens to the intestinal nematode, *Ascaridia lineata,* was lowered by a vitamin-A-deficient diet. They attributed this decrease in resistance to the partial paralysis of the intestine, thus giving the worm a longer time in which to attach itself. Chandler (1943) got no significant difference in the number of worms that became established in rats on a diet deficient in vitamins A, D, and E from that of his controls and stated that the worm is independent of the fat-soluble vitamins in the diet of the host. The rats used by Chandler were depleted for only two days before being infected, which might account for his results.

In the case of cestodes, the partial paralysis of the gut would apparently have no effect on the establishment of the worms if evagination of the scolices had not occurred. From work done on the evagination of tapeworm cysts *in vitro* by the use of bile salts (Edgar, 1941), it would seem that the proper secretion of bile in the host would be necessary for the normal evagination of the cysts in the intestine. It is possible that a vitamin-A deficiency interferes with a normal secretion of bile. If it did, the evagination of the scolices might not occur except near the entrance of the bile duct into the intestine, where the concentration of the bile would be the highest, other cysticercoids passing on through the intestine without evaginating. Such cysticercoids as did evaginate would then be established in a favorable anterior position as postulated above.

The smaller number of worms present in the rats on a G-complex-deficient diet may be due to their being eliminated after initial establishment, either because of a tendency to shift position in an unfavorable environment, or to a weakening of the musculature.

SUMMARY

1. To test the effects of the presence or absence of various vitamins and vitamin combinations on the establishment and growth of *Hymenolepis diminuta* in rats, a series of three experiments was made, with no depletion period, 4 days depletion, and 17 days depletion, respectively, prior to infection. Groups of six female albino rats were infected with 10 cysticercoids each and autopsies made on the fourteenth day.

2. The average number of worms per rat was reduced by a diet deficient in either the fat-soluble vitamins or the G complex and was reduced still further when both of these vitamins were missing. It is suggested that the effect of the lack of the fat-soluble vitamins may be due to some disturbance in the secretion of bile, thus preventing the proper evagination of the cysts. The absence of vitamin B_1 has very little, if any, effect upon the establishment of the worms.

3. The experiments corroborate previous evidence that the lack of some factor associated with the G complex in the diet of the host causes a marked stunting in the growth of the worms. This becomes more marked the longer the depletion period of the host prior to infection, suggesting that the necessary component is absorbed from the host's mucous membrane, and is not obtained in adequate amount in a depleted host. The lack of vitamins A, D, and E or B_1 in the diet of the host causes an increase in size of the worms present. It is suggested that this increase in size over that of the controls is the result of the partial paralysis of the intestine, brought about by the vitamin deficiency, thus enabling the worms to become established in a more favorable anterior position. Since the worms grow in a perfectly normal manner in the absence of any vitamin in the diet of the host except something associated with the G complex, even after a long depletion period which should reduce them in the tissues with which the worms are in contact, the possibility of synthesis of these vitamins by the worm is suggested.

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THE FLEA GENUS *THRASSIS* AND SYLVATIC PLAGUE, WITH THE DESCRIPTION OF *T. BRENNANI* N. SP.[1]

GUSTAF F. AUGUSTSON[2]

During the past decade entomologists and bacteriologists of far Western United States have been conducting extensive investigations on the fleas of native rodents and the incidence of sylvatic plague among these animals. Of the reports published, the résumé by Eskey and Haas (1939) gives the most comprehensive analysis of this relationship. In this report, it is indicated that, of the fleas under observation, species of the genus *Thrassis* far exceeded any others. Likewise, incident to sylvatic plague studies, Jellison (1937) found specimens of *Thrassis petiolatus* (Baker) and *Thrassis pandorae* Jellison in much greater abundance than other fleas collected. It is more than a probability that members of the genus *Thrassis* can be closely associated with the occurrence, or possible occurrence, of plague among native rodents.

According to literature, sylvatic plague has so far been observed only in the far Western areas of the United States, but it is of interest to note that Eskey and Haas (l.c.) have suggested the possibility of its incidence east of the Rocky Mountains. Whether it will be recorded may depend in part on the flea fauna of rodents in other areas that are under observation. Recently Hubbard (1943) described the first known *Thrassis* (*T. princei*) east of the Rockies. This flea was taken from the Great Plains grasshopper mouse, but as Dr. Hubbard suggested, its true host is probably the 13-lined ground squirrel of that area. This record, and the one reported on in this paper, indicates that the genus *Thrassis* may in future sylvatic plague observations and studies be as important in other areas as it has been proven to be in the far West. Following is a list of the known species of the genus and their usual hosts. To date, there are sixteen (16) valid species and three (3) questionable subspecies, all from the Western Nearctic:

Thrassis acamantis (Roths.), ex marmots.
Thrassis arizonensis arizonensis (Baker), ex antelope ground squirrels.
Thrassis arizonensis littoris (Jordan), ex round-tailed ground squirrels.
Thrassis bacchi (Roths.), ex 13-lined ground squirrels.
Thrassis desertorum Stewart, ex antelope ground squirrels.
Thrassis fotus (Jordan), ex 13-lined ground squirrels.
Thrassis francisi (C. Fox), ex Yakima ground squirrels.
Thrassis gladiolis gladiolis (Jordan), ex round-tailed ground squirrels.
Thrassis gladiolis caducus (Jordan), ex antelope ground squirrels.
Thrassis howelli howelli (Jordan), ex marmots.
Thrassis howelli utahensis Wagner, ex marmots.
Thrassis jellisoni Hubbard, ex Oregon ground squirrels.
Thrassis pandorae Jellison, ex Montana ground squirrels.
Thrassis pansus (Jordan), ex ground squirrels and skunk.
Thrassis petiolatus (Baker), ex red digger ground squirrels.
Thrassis princei Hubbard, ex grasshopper mouse (?).
Thrassis rockwoodi Hubbard, ex Oregon ground squirrels.
Thrassis spenceri Wagner, ex marmots.
Thrassis stanfordi Wagner, ex marmots.

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Although Eskey and Haas (1.c.) have demonstrated transmission of native rodent plague experimentally with some species of *Thrassis,* much needs to be done for the remainder. Some points of interest in regard to *Thrassis* and sylvatic plague

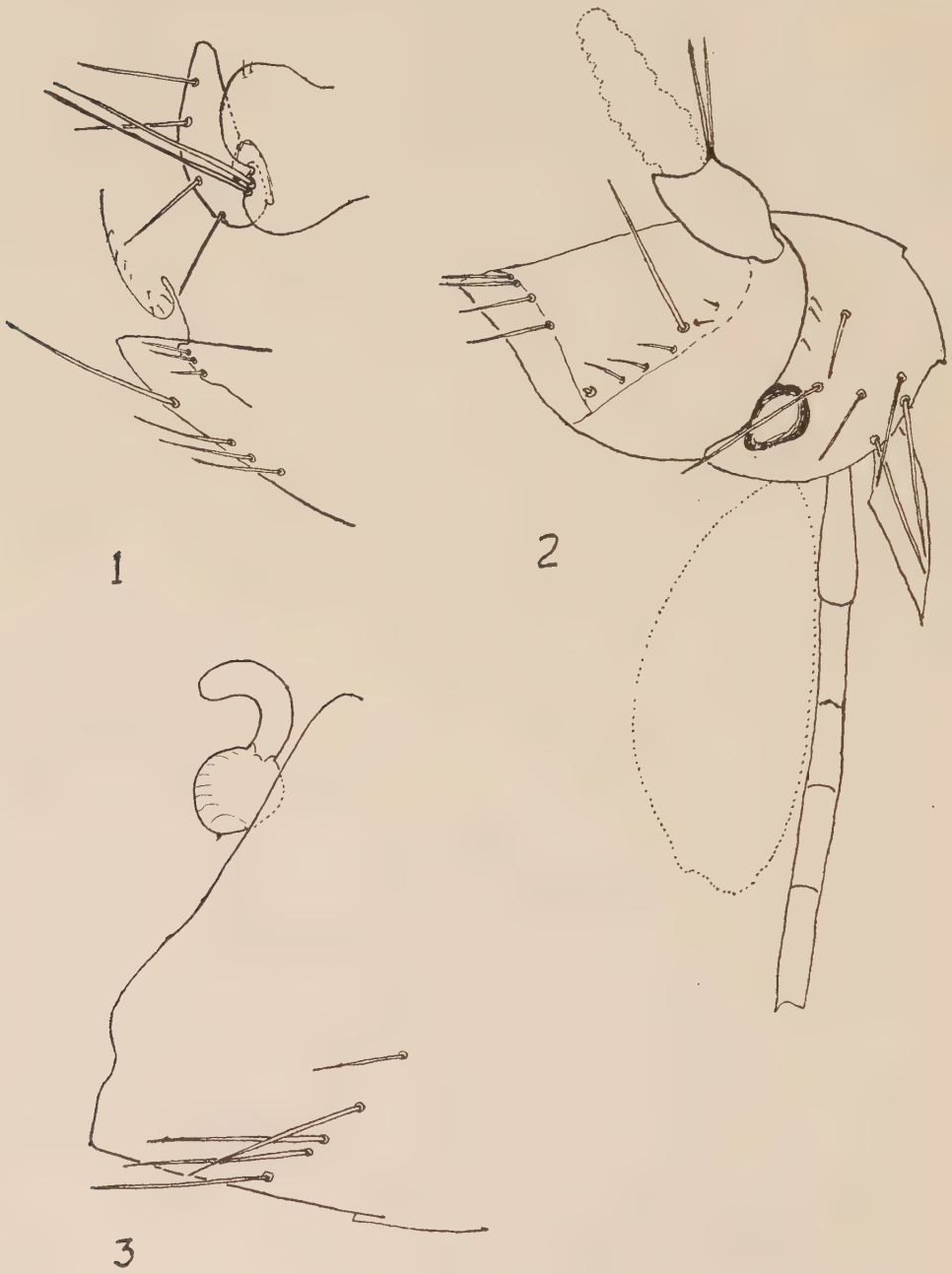

PLATE I

FIG. 1. *Thrassis brennani* Aug., finger, clasper, sternites IX and VIII of holotype male.
FIG. 2. *Thrassis brennani* Aug., head, holotype male.
FIG. 3. *Thrassis brennani* Aug., spermatheca, sternite VII of allotype female.

transmission are the host specificity and life habits of the genus. Occasionally, they will be found on other small mammals associated with the host, as pointed out by Hubbard (1.c.), but cannot be collected consistently from such associates. Yet they should be regarded as ideal vectors because they are present in such great numbers and can apparently withstand periods off the host and be found in or around its burrow. In a colony of ground squirrels, for instance, it would not be unusual to expect that each individual *Thrassis* may in its lifetime have an opportunity of feeding on a number of members of the colony. The fact that these fleas can be found at burrow entrances has been taken advantage of by many collectors. By rolling a strip of cotton on the end of a stick and poking it into burrows, a number of fleas can be captured. The writer has made use of this method often but has not been able to obtain species of *Thrassis* as readily as specimens of *Hoplopsyllus anomalus* (Baker) and *Diamanus montanus* (Baker). It may also be of interest to note here that the writer has never been attacked by any *Thrassis,* nor are there any certain records that other workers have, but he has frequently been so by *Hoplopsyllus anomalus* (Baker), and has recorded (1943) one positive record of *Diamanus montanus* (Baker) attacking man. It can be assumed, however, that species of *Thrassis* are of importance in the transmission and dissemination of sylvatic plague among their native hosts.

With the above information in mind, the following description of a new *Thrassis* is of added interest. This record definitely establishes the genus *Thrassis* east of the Rockies, and also indicates it can be readily taken from the native rodents of this area. During the past year, Dr. J. M. Brennan, while conducting field activities, incident to Bullis fever work, for the Post Surgeon, Fort Sam Houston, Texas, in conjunction with the U. S. Public Health Service, has had the opportunity of collecting ectoparasites from various mammals and birds of this area of the state. From the 13-lined ground squirrel, taken on the grounds of Fort Sam Houston, Dr. Brennan obtained the following flea, which is described and named in his honor.

Family Dolichopsyllidae

Thrassis brennani n. sp.

Holotype male

Head: Frontal notch high, accuminate; preantennal region with two (2) rows of bristles, the upper row with three (3) bristles, the one on genal margin much larger than other two (2), the lower row with three (3) bristles, the center one half the size of the two (2) outer; eye elliptical, not heavily pigmented; maxillae sharply accuminate; labial palpi five (5) segmented, longer than fore-coxa; postantennal region with two (2) large bristles on margin of antennal groove (the posterior bristle broken in type), five (5) smaller bristles also along margin.

Thoracic and abdominal segments: Pronotal ctenidium of eighteen (18) slender spines; tergal teeth present as in other members of the genus; a single, large antepygidial bristle; tergite VIII reduced, cone-shaped; process of clasper rounded, not reaching apex of finger, with few small setae on apical margin; finger with inner margin broadly angulate, apex narrow, posterior margin evenly rounded with four (4) large bristles; ventral lobe of sternite IX short, shallow, without modified bristles, three (3) normal bristles in a row at apex; sternite VIII sharply pointed, with one (1) large bristle well down from apex, followed by three (3) smaller bristles; penis short, broad near middle, spring without one-half of a full turn.

Legs: Tarsus V with five (5) distinct lateral plantar bristles; coxa III rather broad, short; chaetotaxy as in other members of the genus.

Allotype female

Head: Frontal notch not as distinct as in male; preantennal region with two (2) rows of bristles, the upper row with two (2), sub-equal, one near antennal groove, one on genal margin, lower row with three (3) bristles, the middle smaller than the two (2) outer; remainder of head as in male.

Thoracic and abdominal segments: Pronotal ctenidium as in male; tergal teeth present, as in other members of the genus; two (2) large, equal antepygidial bristles; style short, not twice as long as greatest width; sternite X angulate with many large bristles; sternite VII variable, with indication of a shallow sinus (that in type injured), the upper lobe smaller than the lower, with five (5) bristles in a row, the upper one smaller than other four (4); spermatheca globular, the arm not swollen distally.

Legs: Tarsi and rest of legs as in male.

Holotype: A male, collected by Dr. J. M. Brennan, Fort Sam Houston, Bexar County, Texas, 8 April 1943, from *Citellus tridecimlineatus texensis.* Deposited in the U. S. National Museum, Washington, D. C.

Allotype: A female, collected and deposited as above.

Paratypes: Five (5) males, thirteen (13) females collected as above, retained in the Eighth Service Command Laboratory, Fort Sam Houston, Texas.

Type host: Citellus tridecimlineatus texensis.

Type locality: Fort Sam Houston, Bexar County, Texas.

Remarks: This new species is close to *Thrassis princei* Hubbard, from which it can be distinguished on the character of the modified segments in the males only, the females of *T. princei* are unknown. Host determination is that of Captain W. J. Hamilton, Jr., Sanitary Corps, formerly at Headquarters, Eighth Service Command.

The writer wishes to express appreciation to his Commanding Officer, Colonel Harvey R. Livesay, M.C., for his constant interest and encouragement in the preparation of this paper.

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CITTOTAENIA SANDGROUNDI, A NEW ANOPLOCEPHALID CESTODE FROM A JAVANESE TREE DUCK*

HELEN EDITH DAVIS

The tapeworms of this study are one of several lots collected in the Dutch East Indies by Dr. J. H. Sandground (formerly Curator of Helminthology, Museum of Comparative Zoology, Harvard College) and kindly presented to Dr. Hughes. The label with the worms indicates that they were taken from *Dendrocygna javanica* (Horsfield) in Batavia in October 1938 and were preserved in seventy per cent alcohol.

The specimens, consisting of two fragmentary strobilae, only one of which has a scolex, were stained in borax carmine and mounted *in toto* in Clarite.

Cittotaenia sandgroundi n. sp.
(Figs. 1–11)

Description: The strobila with scolex has 332 proglottids, none of which are gravid, and is 65 mm long; the other has 288 segments, some of which are partially gravid, and is 71 mm long. Maximum width 3.72 mm. Margins moderately serrate. Body rather thick—no portion of either worm being sufficiently thin for satisfactory study under oil-immersion objective. Scolex (severely flattened under cover-glass pressure) 120 μ long to rear margins of suckers and 243 μ wide. An unarmed rostellum occurs; it comprises recognizable cortical and medullary regions and is 117 μ long and 63 μ wide. Suckers 81 (78–87) × 71 (67–75) μ. Neck about 394 μ long (measured from rear margins of suckers to first distinct line of segmentation) and 330 μ in minimum width.

Proglottids clearly demarcated, all many times wider than long; the youngest 28 × 398 μ; mature ones trapezoidal, 338 (306–360) μ long, 2.80 (2.70–2.86) mm wide anteriorly, and 2.96 (2.84–3.04) mm wide posteriorly; gravid ones 380 (370–400) μ long, 3.23 (3.20–3.42) mm wide anteriorly. and 3.47 (3.35–3.64) mm wide posteriorly. Two pairs of longitudinal excretory canals, situated about one-fourth width of strobila from respective lateral margins—ventral canals very broad, slightly undulant, and vaguely discernible; dorsal ones slender, more serpentine, clearly defined, and situated almost entirely in regions directly dorsal to the ipsilateral ventral canals; no transverse commissures observed.

Genital apertures paired, marginal, far anteriad, and not salient. Genital atria somewhat cup-shaped, largest near their medial sides; 71 (64–78) μ long laterally, 84 (78–90) μ long medially, and 65 (45–75) μ deep in proglottids 179 to 184 from scolex.

Protandrous. Reproductive systems completely paired except for a common uterus. General loci of gonads roughly trisect width of segments. Genital ducts dorsal to excretory canals. Lateral portions of vagina and cirrus pouch in extreme anterior portion of segment. Postero-peripheral margin of each proglottid so overlaps anterior margin of the next as to obscure the vagina and cirrus pouch of the latter. Female genital pore ventral to male pore and almost impossible to identify except in segments having the margins turned up so as to show the cirrus pouch and vagina in optical cross section.

Unless otherwise stated the measurements listed below for reproductive organs were taken from ten mature proglottids.

Ovary, coarsely and irregularly lobed; anteroventral to testes; 111 (90–135) μ in maximum diameter. Vitellarium also irregularly and coarsely lobed, immediately behind ovary, and about half as large as that organ in average diameter. Mehlis' gland immediately dorsal to ovary. Common uterus in mature segments very narrow, thin walled, and comprised of a rather straight transverse canal located somewhat anterior to middle of segment and traversing almost its entire width dorsal to excretory vessels and ovaries, and a pair of short stems leading directly posteriad

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to oötypes; in our oldest segments (only partially gravid) the transverse canal has become unevenly enlarged, ranges from 9 to 82.5 μ in diameter, and contains eggs (10.5–13.5 μ in diameter) in scattering groups of varying numbers. Leading inward from the atrium the vagina apparently follows the posterolateral surface of the distal two-thirds of the cirrus pouch; thence it passes forward by a gentle S-curve under the pouch to the anterior surface of the segment; now, as a more clearly visible narrow canal, it crosses over the excretory vessels; next, describing a gentle arc, it reaches the oötype. A portion of the vagina between the excretory tubes and the ovary is somewhat dilated as a seminal receptacle 115 (97–135) × 31 (28–48) μ.

Testes, spheroidal; usually four, rarely as few as two but never more than four observed; variously (often somewhat transversely) grouped dorsoposterosublaterad to ovary; 60 (45–75) μ in maximum diameter. Vas deferens, formed by the union of short vasa efferentia that arise on anterodorsal surfaces of testes, passes forward over or near the ovary and follows course of vagina anterolaterad to region dorsal to excretory canals where it expands into a small spheroidal external seminal vesicle [50 (43–57) μ in diameter in 5 examples] which is connected with the cirrus pouch by a short, tightly folded S-curve. Cirrus pouch, very prominent, elongate, subcylindrical, nearly straight in mature segments, and heavily muscular; with cirrus only slightly protruded, 575 (447–600) × 105 (99–112) μ; extends directly mediad almost to, rarely a little beyond, poral excretory vessels. There are apparently two internal seminal vesicles arranged in series in the medial end of the pouch, the proximal one [60 (57–75) × 32 (27–45) μ in seven specimens] being the smaller and more clearly defined. Cirrus stout and very prominent; subcylindrical and tapering gradually from broad base to truncate tip; densely covered with fine spines which vary in form from comparatively stout hooklets 5–6 μ long over most of the organ to hair-like setae up to 18 μ long about its base; 167 (156–180) μ long, 58 (46–71) μ wide at base, and 24 (21–27) μ wide at tip, where fully protruded in subgravid segments; never seen to be fully retracted even in very young segments. The fully exerted cirrus, observed only in our oldest segments, is seated in a thick-rimmed collar-like receptacle which seems to represent a portion of the floor of the atrium which has been partially everted and rolled outward about its base and set off by a constriction.

Host: Dendrocygna javanica (Horsfield).

Locality: Batavia, Java, Dutch East Indies.

Cotype specimens: Several microscope slides bearing whole mounts of one scolex and fragments of two strobilae in U. S. Nat. Mus. Helm. Coll., No. 36899.

The genus *Cittotaenia* Riehm, 1881, contains tapeworms parasitic as adults in birds and mammals. Baer (1927) recognized nine valid species; apparently no new forms have been described subsequently. The new worm was studied in comparison with Baer's descriptions of, and found to differ significantly from, all of the old species. Differing from *Cittotaenia sandgroundi* n. sp. with regards to features mentioned, the five previously known avian species are as follows:

(1) *C. africana* Joyeux and Baer, 1927, from *Bucorax* sp. in Africa has the dorsal excretory vessels connected by a transverse canal in each segment, posteriad genital pores, 200 to 240 testes, and paired uteri with the median end of each uterus slightly bifurcate;

(2) *C. avicola* Fuhrmann, 1897, from *Anas* sp. in "Suisse?" has posteriad genital pores and 120 to 140 testes;

(3) *C. kuvaria* (Shipley, 1900) Fuhrmann, 1901 (= *C. columbae* Skrjabin, 1915, according to Baer (1927)), from columbiform birds in Oceania has midlateral genital pores, 100 to 260 testes in 5 to 6 horizontal rows, unarmed cirri, and a uterus with 6 to 8 posteriad diverticula;

(4) *C. psittacea* Fuhrmann, 1904 (= *Paramoniezia suis* Maplestone and Southwell, 1923, according to Baer (1927)), from *Stringops habroptilus* and *"Sus scrofula* L. ??" in New Zealand and Australia has about 200 testes and the uterus located posteriad to testicular field; and

(5) *C. rhea* Fuhrmann, 1904, from *Rhea americana* in South America has about 100 testes, posteriad genital pores, and a diverticulate uterus.

In the description of the family Anoplocephalidae Kholodkowski, 1902, Meggitt (1924), Baylis (1929), Joyeux and Baer (1936), and Craig and Faust (1940)

all describe the scolex as being unarmed and *without a rostellum.* The occurence of a rostellum in the new species is therefore a very unusual if not unique feature for the family. The small number of testes distinguishes the new worm from all other known species of *Cittotaenia.*

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Explanation of Plate, p. 244

All figures concern *Cittotaenia sandgroundi* n. sp. and were made with the aid of a camera lucida.

Abbreviations

a—genital atrium
c—cirrus
d—dorsal excretory canal
e—ventral excretory canal
f—vas deferens
k—external seminal vesicle
m—Mehlis' gland
n—internal seminal vesicles
o—ovary
s—seminal receptacle
t—testes
u—uterus
v—vagina
y—vitellarium

Fig. 1. Mature proglottid; about one-half width of segment represented. Ventral view.

Fig. 2. Portion of partially gravid segment. Ventral view. Testes no longer clearly discernible at this stage.

Fig. 3. Scolex. Dorsal view.

Fig. 4. Gonads, vitellarium, and proximal portions of associated ducts. From a mature segment. Dorsal view.

Fig. 5. Anterolateral corner of a partially gravid segment showing the genital atrium partially everted and forming a collar-like receptacle about the base of the fully protruded cirrus.

Figs. 6–11. Six sets of gonads showing variations in the arrangement of the testes which are represented in heavy outline. Dorsal views.

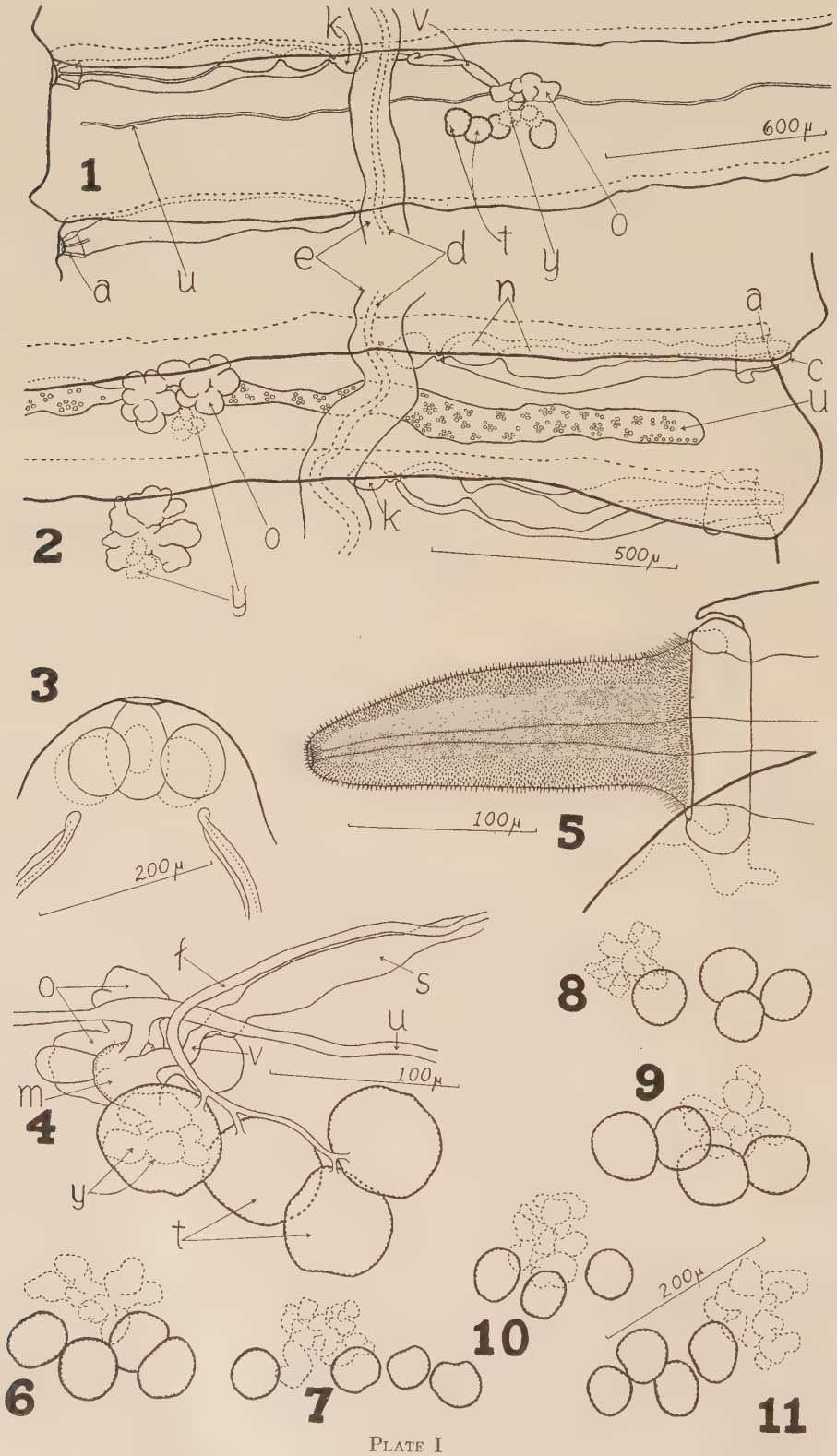
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PLATE I

THE MORPHOLOGY AND LIFE HISTORY OF *FOLEYELLA DUBOISI* WITH REMARKS ON ALLIED FILARIIDS OF AMPHIBIA

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Rana esculenta ridibunda from Lake Huleh, North Palestine, is often infected with a filaria which we identify as *Foleyella duboisi* (Gedoelst, 1916) Yorke & Maplestone, 1926. The intensity of infection varies between 3 and 50% in samples brought from different parts of the shore; the cause of this variation is unknown. The adult worms are situated in lymphatic sinuses, mostly in the subvertebral one, or between the serous sheaths of the mesentery. Occasional specimens are found in other sites, i.e., between the lungs, near the anus, etc. Their number in a single frog varies from 1 to 34 but usually 5–6 are found. Females always predominate. Microfilariae are found in the blood, lymph and all tissue fluids and they may easily be observed in the capillaries of the web of the frog's foot stretched under the microscope. Their number apparently depends not only on the number of adult worms present, but also on the duration of the infection. In the early period of infection their number rises to a maximum, but subsequently either larvae are destroyed or the productivity of females diminishes and the microfilariae become less numerous. The presence of only one female may be associated with numerous microfilariae in each drop of blood. The longevity of the adult filariae in frogs kept in captivity is at least one and a half years.

Foleyella duboisi has been hitherto recorded only once, Gedoelst (1916), from a "big toad" (crapaud géant) and an undetermined frog in Leopoldville, Belgian Congo, and is rather well described. The examination of our material showed some deviation from the original description and it is therefore advisable to record our observations.

Both males and females are whitish and smooth and move actively when placed in 0.65% saline. The oral opening is round and is guarded by two insignificant lateral folds of cuticle; true lips are absent. There are four small salient papillae situated sublaterally about 20 microns from the oral opening. The latter is continued into an almost cylindrical oesophagus divided into an anterior, short and narrower muscular portion, and a posterior, wider and longer glandular one. The proportion between these two parts is as 1 : 7 in length. The width of the posterior portion is not uniform, it is slightly narrower in the middle. The part of the intestine which follows the oesophagus is very wide, sometimes almost filling the total width of the body and it gradually narrows posteriorly. On both sides of the anterior extremity, just behind the level of the head papillae, the cuticle forms alae-like fringes, the free edges of which are longer than the line of attachment and therefore the structures are distinctly undulated. These fringes become gradually smoother and narrower and disappear at the level of the beginning of the intestine to reappear again in both males and females in the posterior extremity of the body.

Male: Length 9–12 mm, maximum width (0.2 mm) is at the level of the anterior part of the intestine, and the body gradually tapers towards both ends. The oesophagus is 1.4–1.5 mm long. In fixed specimens the posterior extremity of the body is coiled spirally 2–3 times; the ventral

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surface of this part of the body is covered with transverse rows of minute tubercles which disappear about 0.5 mm from the tip of the tail. At this level the lateral fringes reappear and they have the same undulating appearance as at the anterior extremity. They do not reach the tip of the tail and are broadest in their middle. The cloacal opening is situated 0.05–0.08 mm from the tip of the tail. Just at its sides or a little in front, there is a pair of big, semiglobular sessile papillae followed by three pairs of smaller post-anal papillae almost uniform in size. The examination of many specimens has shown that this arrangement is constant. The spicules are conspicuously unequal in size and shape. One is 0.36–0.52 mm long, thin, round in cross section, wider at the proximal extremity. The other is 0.10–0.12 mm long, wide, slightly bent and has a groove on its concave side. The body is expanded by coils of two long and wide seminal vesicles. The extremity of each seminal vesicle terminates abruptly and is continued into a short, club-shaped testis.

Female: Length 25–33 mm. The body is almost uniform in width, 0.3–0.5 mm, except at both tapering extremities. The cuticular fringes of the anterior extremity are undulated, as in the male, while at the posterior extremity they are very narrow, smooth, gradually disappearing at about 0.05 mm in front of the tip of the tail. The tail is smooth and has no papillae. Oesophagus is 1.5–1.7 mm long. The posterior part of the intestine widens to form an almost globular ampulla. The anus is situated at ca. 0.4 mm from the tip of the tail. The uterine coils are twisted and expand the body, especially in larger specimens, in which they may reach the posterior extremity; in young specimens they do not reach even the anus. The ovejectors are muscular and much narrower than the uterine tubes. They are long and usually unite in front of the intestine to form the vagina which is 2.0–2.5 mm long, coils round the posterior part of the oesophagus and opens at the level between the second and posterior thirds of the latter.

The above description corresponds to the description and drawing of *F. duboisi* given by Gedoelst (1916) except for the number of oral papillae. Gedoelst mentioned 4 submedian papillae around the mouth, in addition to two lateral ones, while our specimens possess only the former. According to Gedoelst in the species observed by him the lateral fringes commence 0.048 mm from the mouth, i.e., practically at the same distance as the two prominent papillae. We presume that Gedoelst misinterpreted the commencement of the fringes for additional papillae. Fig. 1 made from a preparation of the tip of the anterior extremity of the worm illustrates the possibility of such an error.

Microfilariae of *Foleyella duboisi* may easily be observed in blood vessels of the distended web of a frog's foot. They have an active snake-like movement (Fig. 7); when the blood current is artificially stopped they remain active but hardly change their place. When removed from the blood vessel they wriggle vigorously but do not show translatory movement. In citrated blood they remain active at least two days at room temperature.

In general appearance the microfilariae of *Foleyella duboisi* resemble those of *F. brachyoptera* and *F. ranae* as illustrated by Kotcher (1941). They are slender, almost cylindrical, ca. 5 microns wide at their anterior extremity, tapering gradually to the opposite end. The anterior extremity is obtuse and often forms a knob separated from the rest of the body by a slight constriction. There is a very delicate sheath, longer than the body, visible at one extremity or the other. It is practically unstainable and is best seen in living microfilariae. The cuticle is finely striated. The nuclei are crowded and fill up the entire space between the walls. They are of varying sizes, irregularly round and are all similar with respect to staining properties. In the anterior part of the body they are arranged four abreast, behind the cell G_1 three abreast, behind the cell G_4 two abreast and in the tail there are about 10 nuclei in a single row, the last being often 2–3 times longer than others. The cephalic space is as long as wide, is surrounded by a layer of loose nuclei the disposition of which varies in individual microfilariae, and appears to be devoid of cephalic

structure ("Kopfgebilde"). The nerve ring is a constant feature though it is not always conspicuous (in microfilariae of other species of the genus *Foleyella* it has been reported only in *F. striata*). None of the staining methods used by us revealed a structure corresponding to the central viscus (so called "Innenkörper"). However just behind the middle of the body of the microfilaria there is an unstainable area 5–12 microns long, and adjacent to it one or more ordinary nuclei may be seen in close proximity to the wall. Apparently it is a homologue of the central viscus. The G_1 cell is as wide as the body; other G cells are half this size. The anal-pore cell is situated between the G_3 and G_4.

The differential characters of the microfilaria of *F. duboisi* are shown on Table 1 together with the characters of other microfilariae of this genus, so far as they are known from the literature.[1] Unfortunately it is impossible to consider other microfilariae of amphibia for the differentiation of species because their descriptions were made from material fixed and stained by methods which do not give uniform pictures. Even dimensions of length are of little value because most of the authors failed to measure the microfilariae in extended condition. If not killed before fixation by heat or other method, microfilariae shrink on the slide during drying to such extent that they are half or less of their original length. Microfilariae of *F. duboisi* killed in fluid by gentle heating of the slide shrink very little. By using Mayer's haematoxylin, Pappenheim's stain, Azur, Giemsa and other stains we were able to establish the fixed cells and spots necessary for differentiation of species. However, staining alone did not make the study of microfilariae simple because their nuclei are thickly crowded and the whole content of a microfilaria absorbs stain so that the nuclear column often appears like a continuous dark mass obscuring the fixed cells. We found that microfilariae taken by a leech remain in its crop not disintegrated and retain staining properties for at least ten days (many of them are motile after 24 hours). During this time the digestion of microfilariae proceeds very slowly and it affects primarily the loose tissue of the latter but not the nuclei. On Giemsa-stained slides made from the gut contents of a leech on the 3d to 10th days after feeding on the frog, the nuclei and the main cells of the microfilariae remain practically unchanged and appear discrete and much more distinct than in untreated material. The microfilariae appear a little swollen, 7–8 μ wide instead of 5, and their anterior extremity is rounded instead of obtuse. This phenomenon is apparently due not only to slow digestion but also to peculiar preserving properties of the secretion of the leech gut.[2] These results were obtained with an undetermined leech of the genus *Haementeria* and with *Limnatis nilotica* which readily feed on frogs. It is noteworthy that in a dead frog the microfilariae lose their fine structure and after two days no details can usually be made out.

In order to elucidate the life history of *Foleyella duboisi* experiments were performed with mosquitoes, for other species of *Foleyella* are known to be transmitted

[1] We presume that the microfilaria observed by MacFie (1914) in *Bufo regularis* in Nigeria is identical with *Foleyella duboisi*. The approximate formula of this microfilaria, as judged from the drawing of MacFie (No. 2), is as follows: headspace present, NR—12½%, EP—28½%, G_1—65%, G_2–G_4—80%. The only difference between this and our microfilaria is the absence of central viscus. However, this may be explained by improper fixation of the slide.

[2] Mathis & Léger (1911: 100) made a similar observation with human spirochaetae. When ingested by a leech, they were found dead after 5 days and showed morphological details very clearly on Giemsa-stained slides.

TABLE 1.—*Characters of determinable microfilariae of frogs* *

Species	Length in microns	General shape	Cephalic space	in %								
				N.R.	E.P.	E.C.	C.V.	G_1	G_2	G_3	G_4	A.P.
brachyptera	128 (Causey, 1939) up to 184 when heat-killed (Kotcher, 1941)	Almost uniform in width Gradually tapering behind C.V.	? absent	23	31	33	65	73	83	85	85	86
dolichoptera	240 (Causey, 1939) 260–317 when heat-killed (Kotcher, 1941)	Anterior third wider, 2 nuclei abreast, posterior 2/3 narrow, one nucleus abreast	Shorter than width (Causey, 1939) As long as width of the body (Kotcher)	...	19	20	34	55	70	70	71	72
					acc. to Causey, 1939							
				12	18	19	31	50	...	...	...	70
					acc. to Kotcher, 1941							
duboisi	Heat-killed 200–215, in blood films 95–145.	Uniformly wide up to C.V., afterwards tapers gradually	Always present, though inconstant in shape	16–23	27–35	29–36	54–68	66–73	75–82	77–83	79–85	78–84
ranae	90 (Causey) 156–202 heat-killed (Kotcher)	Similar to *M. brachyoptera*	Shorter than width (Causey) absent (Kotcher)	18	27–28	29–31	63–66	71–73	83	85	85–86	88
ranae-sylvaticae	55–97	Width almost uniform	Contains 2 large nuclei	...	...	26	...	37–60	59–72	62–77	71–86	85–91

* Microfilaria of *Icosiella neglecta* which is presumably described in the paper of Desportes, 1941, is not included in the table because the original description was not available. All microfilariae listed here belong to the genus *Foleyella*, except *M. ranae-sylvaticae* Fantham, Porter & Richardson (1942) of which the adult is still unknown. The characters concerning the latter microfilaria have been compiled from two original diagrams.

by these insects. We tried to work with *Anopheles maculipennis* var. *sacharovi* and *A. hyrcanus,* which abound in places where the infection of frogs is common, but these species could not be induced to feed on the frog. *Culex molestus,* which is ubiquitous in Palestine, was then tried with success and all further experiments were done with this species (in Jerusalem, at room temperature, 25–28° C). Some of the mosquitoes were caught in Jerusalem where natural infection of frogs or toads is unknown, and thus infection of mosquitoes could be excluded, but most of them were laboratory bred.

Culex molestus does not feed readily on frogs in the laboratory. Some of the insects were induced to bite under an inverted test tube either after being starved for 3–5 days or by raising the temperature of the frog by an electric lamp. Good results were obtained with mosquitoes which were first allowed to bite man, then to lay eggs and to starve 2–3 days. In a few cases the insects were allowed to bite a man for a few seconds and were then placed on a frog on which they continued to feed. Once started, *Culex molestus* feeds avidly. In some cases mosquitoes, gorged on frogs, discharged almost the whole blood meal from the anus a few minutes after feeding.

Twenty per cent of mosquitoes fed on frogs died during the first 24 hours and a further 6% during the following 24 hours. Most of those which survived 48 hours lived up to 20 days. It seems that the number of microfilariae present in the frog's blood influences the mortality of mosquitoes, the heavier the infection the greater the percentage of mosquitoes which die. After the infective feed, the mosquitoes were kept in muslin cages in damp atmosphere and were fed on raisins. In all ca. 250 mosquitoes were used in our experiments. They were examined by dissection or in serial sections between 1–20 days after feeding. About 35% of mosquitoes became infected. Out of 40 mosquitoes dissected 14 days and more after the feed, 18 contained infective stages. In cases of heavy infection of the frog, hundreds of microfilariae may be ingested by the mosquito. However, only a comparatively small number, and in some cases none, penetrate into the haemocoele of the mosquito to continue their development. Microfilariae removed from the mosquito immediately after feeding usually are not as active as in the frog's blood and some may be motionless. In the intestine of dead mosquitoes some microfilariae remain active for 24 hours.

The developmental stages of the larva of *Foleyella duboisi* were followed in mosquitoes dissected at various intervals. It was found that the development proceeds in the haemocoele of the mosquito and only occasional larvae were found between the bundles of the thoracic muscles. The first sign of development may be observed on the third day. The larva becomes shorter, the pointed tail is slightly bent dorsally, the nuclei become swollen and the formation of inner cells is noticeable. On the fourth day the larva reaches the maximum of shortening (115 μ), it becomes thicker, the tail is distinctly bent dorsally (Fig. 9). Inside the body a mass of cells is formed, the G-cells are still separated, but the G_4-cell and the anal cell unite and become attached to the anal slit. A small excretory bladder appears and an indistinct oral apparatus may be visible. On the 5th day the larva (Fig. 10) becomes almost twice as thick at its posterior extremity as at the anterior one, the tail shrinks and the rectum begins to take shape. The latter consists of two big cells and a cavity between them closed by a plug projecting externally. The excretory bladder

is usually distended and lies across the whole width of the body. The inner cells do not as yet show differentiation, except for the big cuticular cells which, especially in the posterior part of the body, are grouped in six loose rows. The above-described larval stage, Figs. 9–10, will be referred to as the tail-bearing stage.

The 6–10-day larva assumes an appearance which we shall call spur-bearing stage (Fig. 11). It reaches a length of 230–300 μ. The pointed tail has practically disappeared, leaving its cuticle empty, sharply bent dorsally, as a spur-shaped appendage. The inner cells have formed the digestive tube, clearly divided into an anterior longer portion, the oesophagus, and a posterior shorter one, the intestine. The rectum appears like a big bladder and the anus projects externally as a semi-globular papilla. The oral opening projects like a small conical papilla. At this stage the larva shows only very sluggish movement.

After 11–12 days the larva reaches the preinfective stage and prepares to moult (Fig. 12). It is cylindrical, up to 650 μ long and 40 μ wide and it moves actively. Both extremities are rounded. The spur-shaped appendage is directed along the axis of the body. The oesophagus and intestine are fully developed, while the rectum is still bladder-shaped and the anus projects externally. The larva is enclosed in a sheath distinctly visible at its extremities.

After 14 days infective larvae may be found (Fig. 13). They are elongated, 640–840 μ long and 15–22 μ wide. The anterior extremity bears four minute papillae guarding the oral opening. The tail is more or less elongated, smooth or bears a thickening on the tip. The oesophagus may be slightly shorter than half of the body length. It is wide and is distinctly separated from the intestine. The anus is slightly prominent, 33–40 μ from the tip of the tail. These larvae are found up to the 20th day and they are scattered in the haemocoele, the head or in the proboscis.[3]

The maximum number of larvae found by us in an individual mosquito was 28. The development does not always proceed at the rate indicated above. It depends on temperature as well as on some other still unknown factors and may be different in individual mosquitoes of the same batch, which were bred, fed and kept under identical conditions. Often larvae in various stages of development are found in the same mosquito, as is shown in the following examples:

Exp. No. 22.—7 mosquitoes fed on heavily infected frog. After 15 days 3 proved to be infected. In two of them many infective larvae were found scattered in various parts of the body, only one was in the proboscis. In the third mosquito a few spur-bearing larvae were found in the haemocoele and one motile unchanged (!) microfilaria in the intestine, but no infective larvae.

Exp. No. 24.—6 mosquitoes fed on the same frog. After 17 days 3 proved to be infected: (1) 15 infective larvae in the abdomen, thorax and head, 8 spur-bearing larvae and a few intermediate ones in the haemocoele; (2) 3 infective larvae and many spur-bearing ones in the abdomen; (3) 2 infective larvae in the proboscis, 7 in head, abdomen and thorax and a few preinfective larvae in the haemocoele.

It has to be pointed out that *Culex molestus* is rather rare in the locality where *Foleyella duboisi* is common, and, considering the large percentage of infected frogs, it may be assumed that in nature it does not serve as intermediate host. It is probable that some other mosquito which readily feeds on frogs is a more suitable intermediate host and in it the development proceeds more regularly.

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[3] Judging from the observations of Feng (1936) and Kobayasi (1940) on the development of *Wuchereria* and of Highby (1943) on the development of *Dipetalonema* and *Dirofilaria*, it may be assumed that most filariids developing in invertebrates go through four analogous developmental stages of which the first two are commonly included in the general term "sausage-shaped stage."

The life history of *Foleyella duboisi* as interpreted above corresponds to the observations of Causey and Kotcher who in several publications have described the life history of other members of the genus *Foleyella*.

On this occasion it may be remembered that Porta (1912) tried to follow the life history of a frog filaria, which he determined as *"Filaria rubella* Rud.," in the leech, *Hirudo medicinalis*. However, his experiments which are claimed to be positive, were performed in such a manner that the conclusions cannot be accepted. In order to verify the observations of Porta we performed a few experiments in which we let two species of leeches which abound in the locality where *Foleyella duboisi* is common, viz., *Haementeria sp.* and *Limnatis nilotica,* feed on infected frogs. Fourteen days' observation revealed that no migration or development of microfilariae occur. The latter gradually lose their motility during the first 24 hours and remain in the crop of the leech, dead but undigested, sometimes up to 20 days.

TABLE 2.—*List of species of the genus* Foleyella

Name and author	Host	Country
F. americana Walton 1929	*Rana pipiens*	U.S.A.
	R. stenocephala	U.S.A.
F. bouillez n. nom., 1944	*Bufo regularis*	Central Africa
F. brachyoptera Wehr & Causey, 1939	*R. pipiens*	U.S.A.
	R. sphenocephala	U.S.A.
F. candazei (Fraipont, 1882) Seurat, 1916	*Agama colonorum*	Africa
	A. stellio	Palestine
	Uromastix acanthinurus	Algiers
F. chameleonis Kreis, 1938	*Chameleon*	Madagascar
F. chlamydosauri (Breinl, 1913) Yorke & Maplestone, 1926	*Chlamydosaurus kingi*	Australia
F. convoluta (Molin, 1858) Travassos, 1929 (= *F. ranae* Molin, 1858 nec 1856)	*Leptodactylus ocellatus, L. pentadactylus, L. typhonius, Hyla faber, ? Bufo marinus*	Brazil
F. dolichoptera Wehr & Causey, 1939	*R. pipiens*	U.S.A.
	R. sphenocephala	U.S.A.
F. duboisi (Gedoelst, 1916) Yorke & Maplestone, 1929	"Big toad," undertermined frog *Rana escul, ridib.*	Congo
		Palestine
F. furcata Linstow, 1899 nov. comb.	*Chameleon oustaleti*	Madagascar
F. helvetica Kreis, 1934	*R. esculenta*	Europe
F. leiperi (Railliet, 1916) Yorke & Maplestone, 1926 (= *Fil. bufonis* Leiper, 1909)	*Bufo regularis*	Sudan
F. ranae Walton, 1929	*Rana calamitans, R. catesbiana, R. sphenocephala*	U.S.A.
F. scalaris Travassos, 1929	*Leptodactylus ocellatus*	Brazil
F. striata (Ochoterena & Caballero, 1932)	*Rana montezumae*	Mexico
	R. pipiens	
F. vellardi Travassos, 1929	*Bufo marinus*	Brazil

THE GENUS *Foleyella* AND ITS SPECIES

Table 2 lists 16 species of the genus *Foleyella*. It was compiled from the literature but the following changes have been considered:

1. *F. bouillezi* nom. nov. is given to an unnamed filaria described by Bouillez (1916) as parasite of *Bufo regularis* in Central Africa. It resembles *F. convoluta* and *F. vellardi* which are insufficiently described. However, the different geographic distribution of the latter (Brazil) suggests that these species may not be identical. According to Railliet (1916) *Foleyella bouillezi* (= *Filaria sp.* of Bouillez) is identical with *Foleyella leiperi.* On the other hand, according to Seurat (1916) the latter is identical with *F. bufonis* of Leboeuf & Ringenbach, 1910. Walton (1935) agreed with this identification, but there seems to be no sufficient proof to support it.

2. We regard *Foleyellides striatus* (Ochoterena & Caballero, 1932) Caballero, 1935 (= *Chandlerella striata*) as a member of the genus *Foleyella* (and therefore the genus *Foleyellides* Caballero, 1935, as superfluous).

3. *Filaria furcata* Linstow, 1899, is regarded as belonging to *Foleyella.*

4. *Foleyella agamae* (Rhodain, 1906) Yorke & Maplestone, 1926, and also the "stumpy type' of microfilaria described by MacFie, 1914, from *Agama colonorum,* are regarded (after examination of Palestinian material from *Agama stellio*) as synonyms of *Foleyella candazei.*

5. *Foleyella helvetica* Kreis, 1934, is left in the list; however, as long as its male is unknown and no lateral cuticular flanges are described, its relation to the genus *Foleyella* remains questionable.

A critical comparison of the known specific characters of the members of the genus *Foleyella* suggests that the conception of the latter needs revision. As a matter of fact, not a single species is fully in accord with the existing diagnoses. According to Yorke & Maplestone (1926), this genus is characterized, *inter alia,* by a mouth surrounded by a circle of six small papillae and four papillae more externally . . . oesophagus very short. . . . According to Skrjabin & Shikhobalova, 1936, the head possesses two lateral papillae and eight submedian ones. . . . We propose, therefore, the following revised diagnosis of the genus *Foleyella* Seurat, 1917:

Filariinae: Body smooth or finely striated, or areas on the ventral surface of males in some species covered with small tubercles; mouth without true lips or chitinoid or epaulet-like structures, mouth-papillae present or absent; on the sides of the body the cuticle often forms narrow expansions, resembling flanges in some species, which run the whole length of the body or may appear only near the extremities; in the posterior extremity of male these expansions are widened to form caudal alae. *Male:* various numbers of sessile or pedunculated papillae in front or behind the cloacal opening in addition to small papillae at the tip of the tail in some species; spicules very unequal in size and shape: the right one stout and short, the left one considerably longer and thinner; no accessory piece (gubernaculum). *Female:* uteri amphidelph; vulva at the level of the posterior half of the oesophagus or postoesophageal; microfilariae sheathed, in blood. In connective tissue, body cavity or lymphatic sinuses of amphibia and reptiles. *Type species: F. candazei* (Fraipont, 1882) Seurat, 1917.

OTHER FILARIIDS OF AMPHIBIA

Another filarial genus represented in amphibia is *Icosiella* Seurat, 1917, with the following species:

(1) *I. kobayasii* Yamaguti, 1941, from *Rana limnocharis* in Japan.

(2) *I. neglecta* (Diesing, 1851) Seurat, 1917,[4] recorded from *Rana esculenta* and *R. temporaria* in Europe, from *R. esculenta ridibunda* in North Africa and from *R. tigrina* in Indo-China; vectors *Forcipomyia velox, Sycorax siliacea.*

(3) *I. quadrituberculata* (Leidy, 1856) Walton, 1927 (Synonyms: *Filaria amphiumae* Leidy, 1856, *F. nitida* Leidy, 1856, *?F. Ranae pipientis* "Leidy" of Molin, 1858, *F. solitaria* Leidy, 1856 p.p., *Icosiella solitaria* (Leidy). Recorded from *Rana catesbiana* and *R. pipiens* in U.S.A.

Beside the species of the genus *Foleyella* and *Icosiella* mentioned above, many undetermined or poorly described species of filariae in Amphibia have been recorded in many parts of the world (Table 3). Most of them are probably synonyms of the well-established species. These species inquirendae give additional proof of

[4] Diesing mentioned *Filaria ranae esculentae* Rud. as synonym of this species, apparently owing to common host. As a matter of fact, Rudolphi's species is a *species inquirenda.*

TABLE 3.—*List of records of undeterminable or insufficiently described filariae of amphibia*

Name and author	Host	Country
F. appendiculata Schneider, 1866	*Triturus vulgaris*	Europe
F. bufonis M.C.V. Molin, 1858 (= *F. Eupemphigis marmorati* Molin)	*Eupemphix marmoratus*	Brazil
F. cingula Linstow, 1902	*Cryptobranchus alleganiensis*	U.S.A.
	Megabatrachus japonicus	Japan
F. cochleata Railliet, 1916 (= *F. spiralis* Oerley, 1882, nec Linstow, 1907)	*Helioporus albopunctatus*	Australia
F. columbi Blanchard in Montoya y Flores, 1905 (= *F. columbia* of Linstow, 1907)	Toad	Colombia
F. jubae Seurat, 1916	*Bufo bufo*	Algeria
F. parva Polonio, 1859	*Bufo viridis*	Italy
F. ranae Molin, 1856 : 391, nec : 390	*Hipsiboas faber*	Brazil
F. ranae esculentae Rudolphi, 1819	*Rana esculenta*	Europe
F. ranae esculentae Valentin, 1841	*Rana esculenta*	Europe
F. ranae esculentae Vogt, 1842	*Rana esculenta*	Europe
F. rubella Rudolphi, 1819, nec Dujardin	*Rana esculenta, R. temporaria*	Europe
F. rubella Dujardin, 1845, nec Rudolphi	*Rana esculenta*	Europe
F. sp. of Baylis, 1933	*Rana tigrina*	Java
F. sp. of Causey, 1938	*Rana oesopus*	U.S.A.
F. sp. of Darling, 1912	*Bufo marinus*	Panama
F. sp. of Franchini	*Rana esculenta*	Europe
F. sp. of Leger, 1918	*Bufo marinus*	Fr. Guiana
F. sp. of Leidy, 1852	*Rana pipiens*	U.S.A.
F. sp. of Leuckart, 1876, of Linstow, 1899, and of Blanchard, 1887	Frogs	Europe
F. sp. of Plimmer, 1912	*Bufo marinus*	Brazil
F. sp. of Schneider, 1866	*Triturus vulgaris*	Europe
F. sp. of Schwetz, 1930	*Rana alvilabris, Rana occipitalis, Bufo regularis*	Congo
F. sp. of Walton, 1927	*Amblystoma tigrinum*	U.S.A.
F. unguiculata Linstow, 1906	*Bufo viridis* (Teste Walton)	Europe
Microfilaria bufonis of Leboeuf & Ringenbach, 1910	*Bufo regularis*	Congo
Microfil. sp. of Mathis & Léger, 1911	*Rana limnocharis, R. tigrina*	Tonkin
Microfil. sp. of Wenyon, 1931	*Rana macrodon*	Philippines
Microfil. sp. of Yakimoff, 1916	*Rana viridis*	Turkestan
Microfil. tamborini Mazza & Franke, 1927	*Leptodactylus ocellatus*	Brazil
? *Spiroptera bufonis* Stossich, 1901	*Bufo bufo*	Sicily

widespread occurrence of filariae in Amphibia. It is, however, noteworthy that in spite of almost cosmopolitan occurrence of filariae in Amphibia their distribution is not uniform. They are confined to some particular localities while they have not been recorded from neighboring ones.

SUMMARY

Foleyella duboisi (Gedoelst, 1916) is common in *Rana esculenta ridibunda* in Lake Huleh, North Palestine. The description is given of adults, microfilaria and developmental stages in experimentally infected *Culex molestus*. The study of partially digested microfilariae taken from the crop of a leech has been found to facilitate the recognition of their peculiar anatomical elements. The development of larvae in the mosquito takes as a rule 14 days but is often irregular. During this development five larval stages are distinguished: 1°– microfilaria, 2°– tail-bearing stage, 3°– spur-bearing stage, 4°– preinfective stage, 5°– infective stage. It is suggested that these stages also occur in other filariae and that the so-called sausage-shaped stage of filarial development includes the above-mentioned stages 2–3. A list of filariid parasites of frogs is given and a revised diagnosis of the genus *Foleyella* is proposed.

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Explanation of Plates

Foleyella duboisi (Gedoelst, 1916) Yorke and Maplestone, 1926

Plate I

Fig. 1. Anterior extremity of an adult specimen, cut obliquely.
Fig. 2. Anterior end of an adult specimen; scale = 0.04 mm.
Fig. 3. Anterior extremity of a female.
Fig. 4. Unfolded posterior extremity of a male.
Figs. 5 and 6. Posterior ends of two females with differently developed uteri; scale = 0.1 mm. On Fig. 5, anus is not shown.

Plate II

Fig. 7. Outlines of moving microfilariae.
Fig. 8. Microfilaria from intestine of leech; scale = 10 microns.
Fig. 9. Larva; early tail-bearing stage; scale = 10 microns.
Fig. 10. Larva; typical tail-bearing stage.
Fig. 11. Larva; spur-bearing stage.
Fig. 12. Larva; preinfective stage.
Fig. 13. Infective larva; below, variations of the tip of the tail; scale = 40 microns.

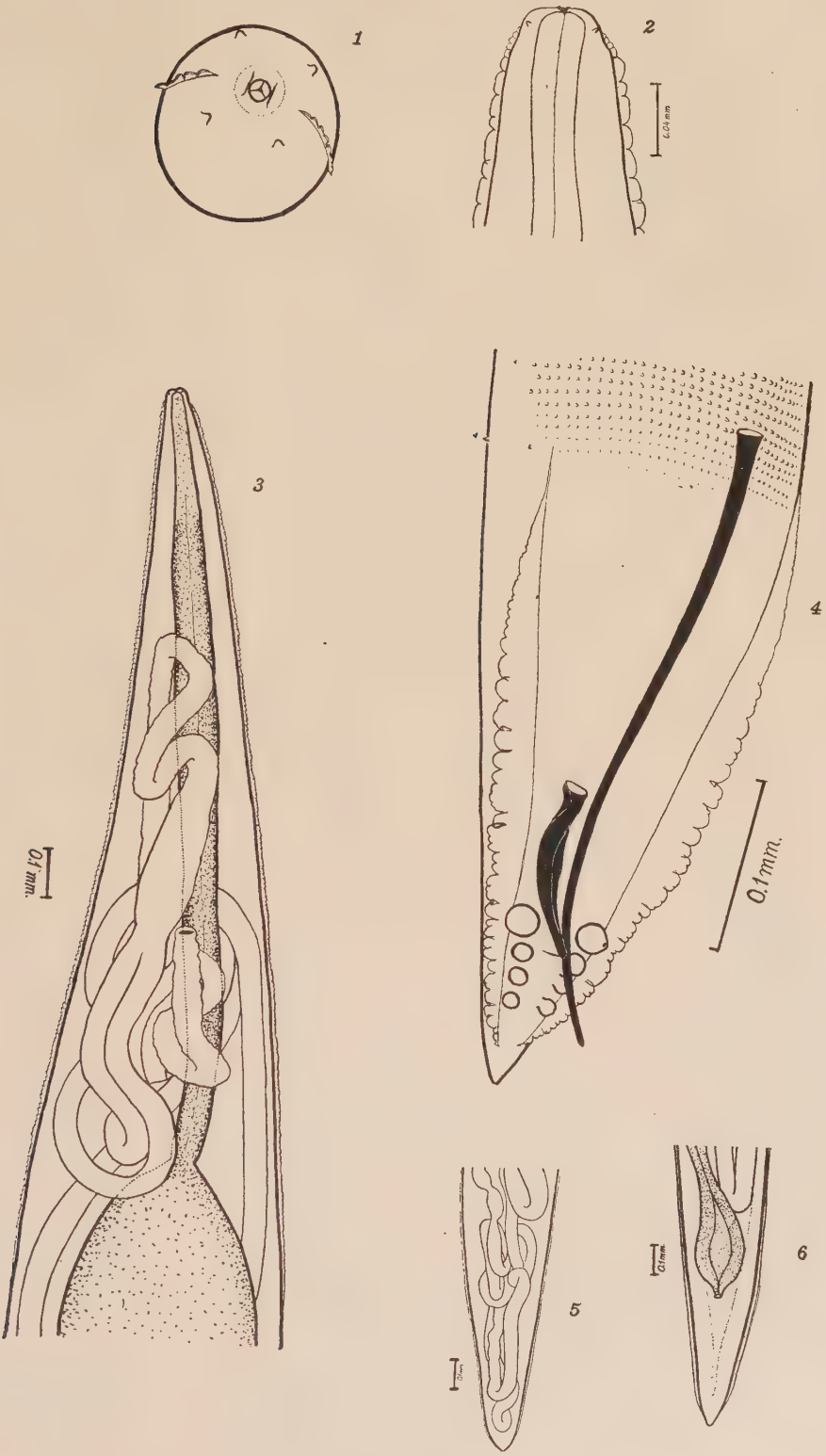
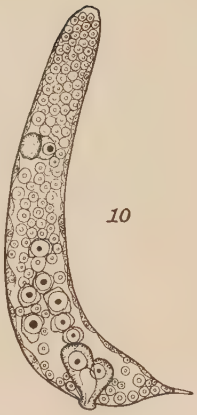
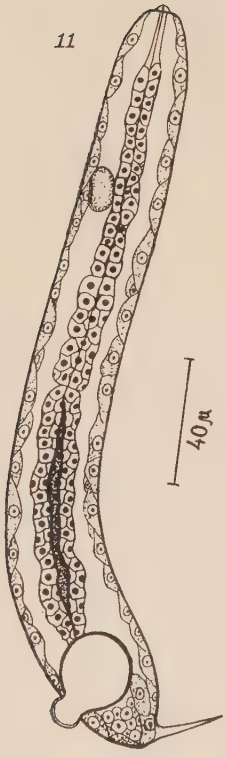


PLATE I





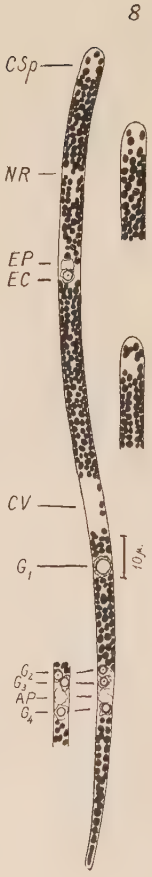


PLATE II

THE LIFE HISTORY OF *NUDACOTYLE NOVICIA* BARKER, 1916 (TREMATODA: NOTOCOTYLIDAE)*

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The greater part of the work on the life history of *Nudacotyle novicia* was undertaken at the University of Michigan Biological Station during the summers of 1939 and 1940. Several phases of the study lack completeness, but since further work has not been possible the data at hand are being made available.

Infection experiments involving the feeding of metacercariae of *Cercaria marilli* Ameel, 1939, to various birds and mammals yielded adult specimens of *N. novicia* from the bile duct of the meadow mouse, *Microtus pennsylvanicus pennsylvanicus*. Formerly this species had been reported only from the small intestine of the muskrat, *Ondatra zibethica zibethica*. Subsequently infection experiments with the snail host provided an insight into the early development of this trematode in the intermediate host.

Daughter redia and cercaria.—The daughter redia and cercaria, *C. marilli,* from the naturally infected snail host, *Pomatiopsis lapidaria,* have been described previously (Ameel, 1939).

The simple sac-like daughter rediae (Fig. 7) occur in the digestive gland of the snail. The body is pigmented and the mouth is terminal, opening immediately into a muscular pharynx. The gut is moderately voluminous and long, extending to the posterior third of the body. It is easily identified under low magnification because of its contents of reddish brown food particles. There seemed to be a relatively small number of daughter rediae per snail. Of a collection of ten infected snails, the number of rediae recovered ranged from 8 to 39 with an average of 24.

C. marilli (Fig. 8) is a trioculate monostome cercaria which occurred in 1.2 per cent of the snails collected at Ann Arbor, Michigan, and in 55 per cent of the snails collected near Marion, Iowa. Both body and tail are capable of considerable extension and contraction. Living cercariae under a supported cover glass measured as follows: body extended, 0.42 mm; contracted, 0.21 mm; tail extended, 0.56 mm; contracted, 0.28 mm. Naturally emerged cercariae have three eyespots but most of the immature cercariae lack the median eyespot. The body is opaque and is filled with brown pigment granules and cystogenous cells packed with rod-like cystogenous material. Spineless posterolateral locomotor organs are present. *C. marilli* resembles the cercaria of *Quinqueserialis quinqueserialis* (Barker and Laughlin) described by Herber (1942). However, the excretory concretions in the excretory bladder of this species are small but numerous, but those of *C. marilli* are large and relatively few.

The metacercaria.—When collections of *P. lapidaria* are placed in small bottles or stender dishes containing water, the cercariae emerge in numbers during the evening, night, and early morning, and encyst after a brief period of activity. Some

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* Contribution from the University of Michigan Biological Station and Contribution No. 238, Department of Zoology, Kansas State College.

cercariae fail to encyst and are found dead upon the bottom of the container. Encystment generally is upon vegetation or the dish, but occasionally cysts are found upon the surface film. If one side of the dish is exposed to light, the cercariae invariably encyst on that side, near the surface of the water. The fact that most of the snails leave the water after a short time or remain partially immersed at the surface indicates that there is a limited period for the emergence of the cercariae after the snails have been placed in the water. Under natural conditions *P. lapidaria* is seldom observed immersed in water but is found living on damp soil near running streams. When fresh collections of these snails were placed in water, there was a good emergence of cercariae overnight, but few cercariae emerged during the succeeding nights. When a continuous supply of metacercariae was desired for infection experiments, the snails were kept on decomposing leaves in shallow dishes. They were removed periodically every week or two and were placed in lettuce cups partially filled with water and fitted into finger bowls in such a manner that when they were covered with glass plates, the snails were sealed in and prevented from wandering far from the water. Large numbers of cysts were collected in this manner. One collection of 558 snails was set out in lettuce cups at 5:30 PM. Four and one-half hours later the lettuce was thickly covered with cysts, and the snails were removed. Three snails were discovered above the surface of the water surrounded by 34, 57, and 159 cysts, respectively. Evidently the cercariae had emerged in the film of water left by the snails and had encysted immediately. Probably this is the common method of dispersal of the cysts upon the vegetation eaten by the mammalian hosts. It was estimated that this collection of snails yielded over 20,000 cysts.

The cyst is disc-shaped with a flat base (Fig. 9). An elevated portion containing the metacercaria is of smaller diameter than the base. The diameter of the elevated portion of ten unflattened cysts ranged from 0.112 to 0.126 mm (av. 0.117 mm) and that the base from 0.168 to 0.182 mm (av. 0.176 mm).

Feeding experiments have demonstrated that the metacercariae are infective immediately after encystment. Evidently, under proper conditions, they are capable of surviving for a considerable period of time. A collection of approximately 1200 cysts kept in the refrigerator on lettuce for 42 days was fed to a meadow mouse, and 976 young worms were removed from the bile duct upon the death of the animal eight days later.

Infection of final host.—Undisturbed cysts on lettuce were fed to three white rats, a pigeon, two rabbits, two guinea pigs, six mallard ducks, two chickens, two muskrats, two canaries, a spotted sandpiper, a bog lemming, and numerous meadow mice. Only the muskrats, meadow mice, and bog lemming, *Synaptomys cooperi cooperi,* became infected.

The adult specimens recovered from the bile duct of meadow mice in most respects resembled *Nudacotyle novicia* common in the small intestine of muskrats from many localities of the lower peninsula of Michigan. That the meadow mouse as well as the muskrat may serve as a natural host of *N. novicia* is indicated from a collection of meadow mice near Marion, Iowa, where the infected snail host is numerous. Two of six trapped meadow mice were infected with this trematode. Three meadow mice trapped in a favorable habitat near Ann Arbor were negative. All the meadow mice collected in the vicinity of the Biological Station have been uninfected.

The bog lemming, which is closely related to the meadow mouse and which shares the same habitat and food, was fed metacercariae to determine whether it could serve as a natural host. The infection was localized in the bile duct. No naturally infected bog lemmings have been collected.

The excysted metacercariae locate exclusively in the bile duct of the meadow mouse within a short time after ingestion of the cysts. The earliest examination of an experimentally infected animal was 18 hours after feeding. After the animal had been fed 300 cysts, 149 worms were recovered from the bile duct and seven from the duodenum. The diameter of the bile duct, which normally is less than a millimeter, increases with the growth of the enclosed trematodes until finally it becomes a conspicuous pink or yellowish sac with a wall so thin that the contents may be discerned through it. The greatest diameter observed was seven millimeters. The average diameter was five millimeters. The usual number of mature worms recovered from an animal surviving the infection was less than 150. As previously mentioned, however, 976 immature worms were removed from the bile duct of an animal that had died eight days after ingesting 1200 cysts. Two hundred and sixty-nine worms with uteri filled with eggs were collected from another animal that had died 17 days after infection. It is assumed that the worms begin to lay eggs at about this age, for a few eggs were first found in routine fecal examinations 18 to 19 days after infection.

The lateral eyespots and scattered patches of body pigmentation are present for some time after the worms become established in the bile duct. All of a collection of 28 nine-day-old worms had scattered remnants of the cercarial pigmentation, but the lateral eyespots remained in 11 specimens. The reproductive system was fully formed but no eggs were present. The pigmentation was more diffuse in 10 eleven-day-old worms, and only one had lateral eyespots. Eggs were observed in 6 specimens; the greatest number in a single specimen being 11. The oldest specimens studied were 64 days old. A marked decline in productivity was evident in these, for the uteri contained but a fraction of the number of eggs observed in worms half their age.

The location of what appeared to be the same species of worm in different parts of the alimentary tract in different but rather closely related hosts caused speculation whether these might be different species. Three wild muskrats one-third to one-half grown were secured through the courtesy of Dr. Lawrence Penner. Two of the muskrats were used as controls. Upon examination, one control was negative, and the other yielded 12 specimens of *N. novicia*. The remaining muskrat, whose feces seemed free of monostome eggs, was fed approximately 600 cysts of *C. marilli*. Four hundred and thirty-seven young adult worms were recovered from the duodenum 28 days later. None was in the bile duct. A trapped adult wild muskrat was fed a total of 2500 cysts over a 12-day period and was examined after the initial feeding. The bile duct was free of trematodes but the duodenum contained 1578. The specimens were in various stages of development, some of the youngest having lateral eyespots. Another adult muskrat caught in the same vicinity as that of the foregoing animal was negative for *N. novicia*. Mature specimens of *N. novicia* from the experimentally infected muskrats in every respect resembled those collected from naturally infected muskrats. Since but two muskrats were fed experimentally and only wild-caught animals were used, the results of this experiment are not regarded

as conclusive. The evidence, however, is sufficient to indicate that *C. marilli* probably develops into *N. novicia* in either the bile duct of the meadow mouse or in the duodenum of the muskrat.

The adult.—Specimens from the meadow mouse (Fig. 1) are on the average considerably larger than those from the muskrat (Fig. 2). Fifteen worms chosen at random from a collection from a meadow mouse measured 0.903 to 1.407 mm (av. 1.123 mm) in length and from 0.462 to 0.833 mm (av. 0.615 mm) in width. Ten specimens from a naturally infected muskrat measured 0.585 to 0.735 mm (av. 0.661 mm) in length and 0.315 to 0.45 mm (av. 0.376 mm) in width. Barker (1916) described *N. novicia* from material from the muskrat. The lengths of his specimens ranged from 0.709 to 0.899 mm, and the widths from 0.501 to 0.657 mm.

Specimens of *N. novicia* from the meadow mouse generally are plump with tapered posterior ends, but those from the muskrat are typically thin dorso-ventrally with concave ventral surfaces and truncate posterior ends.

Since *N. novicia* has been collected from muskrats in the vicinity of the Biological Station and since the known snail host, *P. lapidaria,* has never been reported from the region in spite of extensive collecting, indications are that some other species of snail must also serve as intermediate host. Large collections of local snails have been examined, but none has been found infected with *C. marilli.*

The egg.—Within three to four weeks after infection of the host, numerous eggs appeared in the feces. On several occasions, upon examination of the host, great numbers of eggs were recovered from the bile duct, where possibly they had been trapped.

The eggs (Fig. 3) are amber colored and operculate and bear long polar filaments. Occasionally, atypical eggs with double abopercular filaments were observed in fecal smears (Fig. 4). Barley perceptible miracidia are present in the eggs which have passed from the host.

Fifteen eggs measured in a fecal smear had lengths ranging from 0.019 to 0.022 mm (av. 0.021 mm) and widths from 0.013 to 0.016 mm (av. 0.015 mm). The full length of the egg including the filaments varied from 0.469 to 0.536 mm (av. 0.503 mm).

At two different times eggs were kept in watch glasses at room temperature for a month and a half but none was observed to hatch. It is assumed that possibly they must be eaten by the proper snail host before hatching occurs, though Herber (1942) in his life history studies of two monostomes stated that the miracidia probably hatch and actively penetrate the host. On one occasion a snail was observed to include an egg while ingesting debris from the bottom of the dish.

Infection of snails in laboratory.—Since nothing was known about the rearing of *P. lapidaria* in the laboratory, it was necessary to obtain young snails from a natural habitat and to maintain controls. This species of snail has never been found in the northern half of the Lower Peninsula of Michigan, and so it was necessary to secure young snails from Ann Arbor. On August 3, 1940, fifty young snails of about one to one-and-one-half millimeters in length were placed in uncovered large stender dishes containing moist soil and decomposing leaves to which were added a generous number of eggs collected from adult worms raised experimentally in the meadow mouse. For some unknown reason most of the snails died within a week. No sign of infection could be found in the few survivors examined at this

time. None of 26 surviving controls was infected. Since it was too late for further work at the Biological Station, a supply of eggs and six recently infected meadow mice were taken to Manhattan, Kansas, and another collection of young snails was made at Ann Arbor enroute. The snails were kept in flat pans on damp sod removed from their habitat. Decomposing leaves were added to the pans from time to time.

On October 2, 1940, the mice were sacrificed and eggs recovered. The procedure that followed was identical with that used in the first infection experiment. Since the snails had been in the laboratory for over a month, 50 regarded as controls were examined at the onset of the experiment. Of this number only one was infected.

Mother sporocyst.—Seven days after exposure three snails were examined, but nothing was found that could be positively identified as a mother sporocyst. When two snails were examined seven days later, one mother sporocyst was recovered from one, and two from the other. Over the following week, further examinations yielded more mother sporocysts. In every case, these were found loosely attached along the intestine or floating free in the dish. Only one living mother sporocyst was studied. The remander was fixed in Gilson's, then stained, and mounted for microscopic examination. Unfortunately but three were satisfactory for study. These measured respectvely: 0.130 by 0.087 mm, 0.119 by 0.098 mm, and 0.112 by 0.093 mm. Two mother sporocysts contained four developing mother rediae (Fig. 5), but the other contained five.

Mother redia.—After three weeks, every snail examined yielded rediae in some stage of development. These were located either along the intestine anterior to the digestive gland or in the digestve gland. Examinaton of snails continued until November 18, 1940, when the remaining snails were examined since they were in poor condition. A total of 39 snails was examined of which three possibly were negative. No doubt, because of low temperatures, development had lagged, and no daughter rediae that could be identified positively as such were observed. Some of those recovered toward the latter part of the experiment may have been young daughter rediae. Unfortunately, the material obtained from the experimental infection was such that a careful study of the early generations in the snail was not possible. Further work on this phase of the life cycle is desirable.

The gut of the mother redia (Fig. 6) was readily observed under low magnification, for it was brownish or amber-colored due to its contents of food particles and was relatively large, being globular or elongate in shape. The number of fully formed daughters within any mother redia generally were few. The measurements of 10 fixed and stained mother rediae collected four weeks after infection were 0.14 to 0.248 mm (av. 0.19 mm) in length and 0.084 to 0.109 mm (av. 0.094 mm) in width.

SUMMARY

The life cycle of *Nudacotyle novicia* has been completed in the laboratory by the feeding of encysted *Cercaria marilli* to wild and laboratory raised animals.

The snail host is *Pomatiopsis lapidaria.* The definitve hosts are the meadow mouse and the muskrat. The adult trematode lives in the bile duct of the meadow mouse and in the small intestine of the muskrat.

Mother sporocysts and mother rediae were recovered from young *P. lapidaria* exposed to the eggs of *N. novicia.* These forms are described.

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Explanation of Plate I

(All drawings made with aid of camera lucida)

Fig. 1. An adult specimen from bile duct of meadow mouse.
Fig. 2. Outline of adult specimen from duodenum of muskrat.
Fig. 3. Normal egg.
Fig. 4. Egg with two abopercular filaments.
Fig. 5. Mother sporocyst.
Fig. 6. Mother redia.
Fig. 7. Daughter redia.
Fig. 8. Mature cercaria.
Fig. 9. Encysted metacercaria.

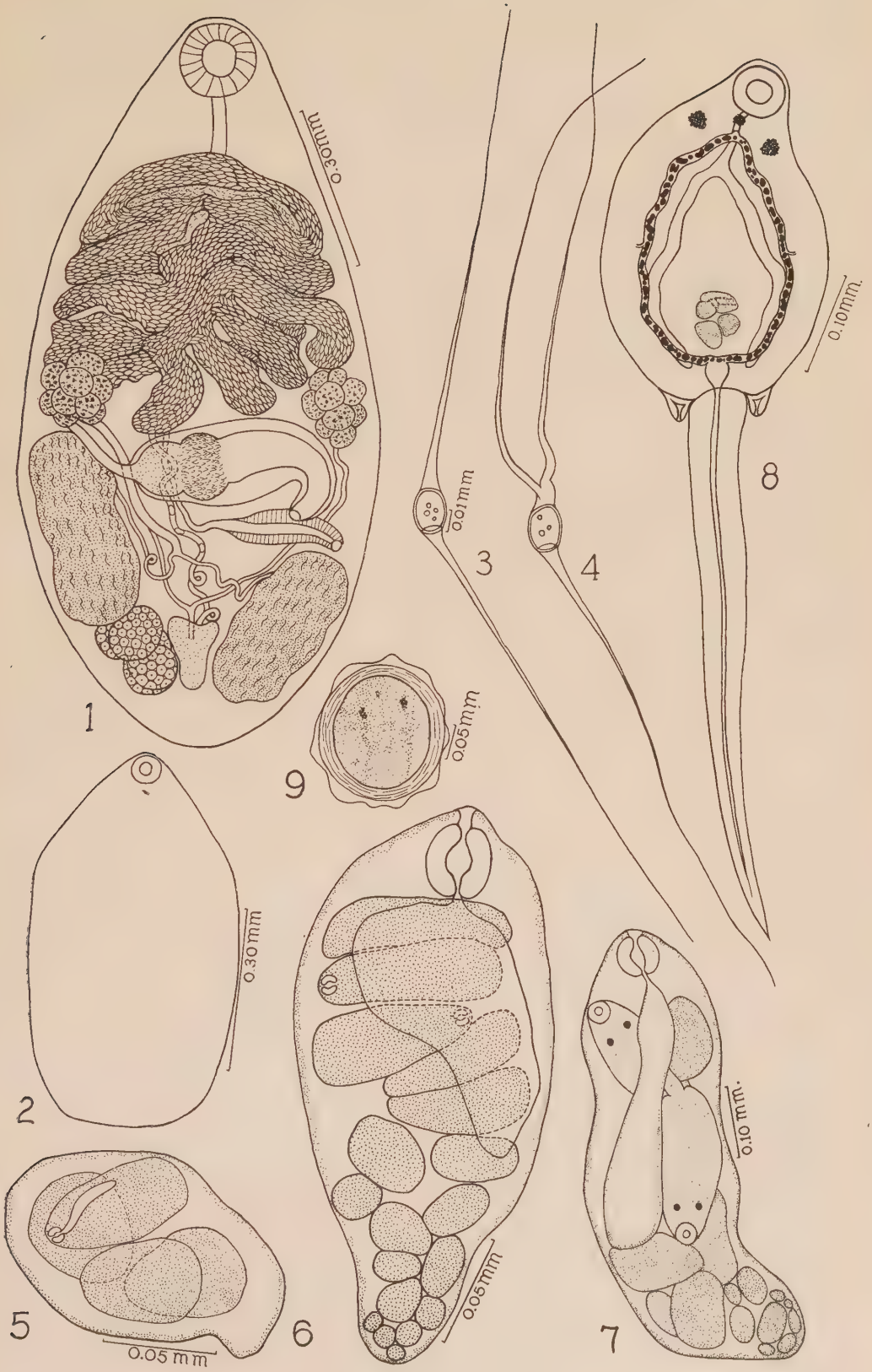

PLATE I

STUDIES ON *CERCARIA SZIDATI* SP. NOV., A NEW FURCOCERCOUS CERCARIA OF THE VIVAX TYPE

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INTRODUCTION

A new longifurcous, pharyngeate cercaria of the Vivax type has been found to develop in *Campeloma* sp. collected from the Tippecanoe River, near Lafayette, Indiana. Although about fifteen species of Vivax larvae have been described, *C. kentuckiensis* Cable, 1935, has been the only fresh water representative of the group reported from the United States. Cable (1938) extended his brief description of this species and gave a comprehensive review of the group in a paper to which the reader is referred. The present species is named in honor of Professor L. Szidat who found a very similar cercaria in East Prussia and determined it to be the larva of *Linstowiella viviparae* (von Linstow, 1877).

Snails were collected during the summers of 1942–43 and examined for emerging cercariae which were studied alive, both unstained and with the aid of supra-vital dyes. Measurements were taken from larvae killed in hot 10% formalin because living cercariae were so active that they could not be measured without excessive cover-glass pressure. A few infected mollusks were crushed to obtain sporocysts and observe the development of cercariae. This investigation was carried out under the direction of Dr. R. M. Cable, whom I wish to thank for his interest, criticism, and suggestions.

OBSERVATIONS

Description of *Cercaria szidati* sp. nov.

(Figs. 1 and 2)

Specific diagnosis: Longifurcous pharyngeate cercaria with the characters of the Vivax group. Measurements in mm of ten specimens: body length 0.386–0.495 (av. 0.437), width 0.172–0.248 (0.205); length of tail-stem 0.694–0.819 (0.742), width 0.086–0.106 (0.098); length of tail furcae 0.429–0.532 (0.471); length of oral sucker 0.076–0.093 (0.087), prepharynx 0.007–0.013 (0.012), and pharynx 0.02–0.033 (0.027). Body pyriform, flattened, and spinose except ventral surface of posterior body region. Tail attached dorsally; tail-stem and furcae spined and with delicate hair-like processes; furcal fin-folds absent. Intestine prominent, with tortuous ceca extending to level of excretory vesicle. Excretory system typical of the Vivax group, the vesicle receiving two median and two lateral ascending tubules; median tubules converge anterior to a prominent mass of nuclei (probably the primordium of the holdfast organ), and fuse to form a single median tubule which extends anteriad to join a cross-commissure connecting the pair of lateral ascending tubules. Slightly posterior to this level, each lateral tubule receives the main collecting tubule which extends posteriad a short distance before dividing into anterior and posterior secondary tubules. Each anterior tubule receives the capillaries of two groups of three flame cells; each posterior tubule is joined by the capillaries of three similar groups and extends into the tail-stem where it receives an additional group of three flame cells; the excretory formula accordingly is $2[(3+3)+(3+3+3+3)]$. Develop in sausage-shaped sporocysts in the mantle region of the molluscan host.

Host: Campeloma sp.
Locality: Tippecanoe River, Indiana, U.S.A.
Incidence of infection: 8.0%.

The cercaria exhibits no decided phototropism and does not react to shadows. However, it responds instantly to the slightest movement of the water by swimming

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in a rapid, jerky manner and then stops suddenly at or near the surface. The larva spends much of the time at rest with the tail flexed, usually to the right, so that the distal part of the tail-stem is about perpendicular to the body axis. Before death, cercariae frequently creep over the bottom of the dish with rapid inchworm movements, occasionally lashing the tail violently, but not rising. Decaudation is frequently observed, and shortly after separating from the body, the tail becomes vacuolated and contracts with the furcae rolled into a tight spiral. The larva will attempt to penetrate anything in its path, often forcing its way into vaseline sealing the preparation. After emergence, the cercariae live from 12 to 24 hours, rarely longer.

Neutral red has a selective action on the glands in the penetration organ and fore-body, the intestinal ceca, and the Island of Cort, while the nuclei of the body and tail are less intensely stained.

Only sporocysts producing cercariae have been observed in the present study (Fig. 2). They are provided with characteristic circular muscle bands and move very actively when dissected from the snail. Unlike the sporocysts of *C. kentuckiensis* which occur in the digestive gland of the snail, those of *C. szidati* are found only in the mantle region and adjacent portions of the visceral mass. The excretory pattern of the sporocyst was not determined, but enough was observed to show that it was very complex.

Cercaria kentuckiensis, or a very similar species, occurs in *Goniobasis depygis* from McCormicks Creek, Indiana, and has been available for comparison with *C. szidati.* These species are similar in respect to body shape and spination and swimming activities. In both, the muscle fibers of the tail-stem have a distinctly striated appearance under high magnification, and the tail-stem and furcae are spinose, although Cable (1938) did not find spines on the tail-stem of *C. kentuckiensis.* These species differ in many respects, however. *C. szidati* is much the larger of the two, lacks furcal fin-folds, and has less tortuous intestinal ceca which, furthermore, contain no concretions resembling those observed in the intestine of *C. kentuckiensis.* Also, the body of *C. szidati* is less glandular, especially in the lateral and posterior regions, making it much easier to trace the flame cell pattern than in *C. kentuckiensis.* Although Cable (1938) found 14 pairs of flame cells in the latter species, he did not observe the capillaries and their connections. Since the flame cells of *C. kentuckiensis* are much smaller than those of *C. szidati* and are extremely difficult to observe, it seems probable that both species may have the same pattern, viz., 15 pairs of flame cells in the body. Although the flame cell pattern may be identical in these species, several differences in their excretory systems are apparent upon careful study. The median and lateral ascending tubules of *C. szidati* lack concretions, and the anterior ends of those tubules are not extended as in *C. kentuckiensis.* Also, the median pair forms a single loop embracing the primordium immediately anterior to the excretory vesicle, instead of two loops as in *C. kentuckiensis.* The primordium of the ventral sucker, which is enclosed by one loop in the latter species, appears to be undeveloped in *C. szidati.* Another difference in the excretory system of the two species is provided by the furcal tubules which are always distinct in *C. szidati* and situated near the middle of the furcae. Pores at the tips of the furcae were observed in immature specimens, but could not be demonstrated in larvae emerging from the snail.

Of the reported species of cercariae belonging to the Vivax group, *C. szidati* resembles most the cercaria of *Linstowiella viviparae* (von Linstow) as described by Szidat (1933). However, these species differ in respect to body size and the proportionate lengths of the tail-stem and furcae, and their excretory patterns are quite dissimilar. According to Szidat, the larva of *L. viviparae* penetrates and encysts in the Molluscan intermediate host. In the present study, specimens of *Campeloma sp.*, both infected and uninfected, and other species of snails were exposed to *C. szidati* for prolonged periods, but no metacercariae were recovered in subsequent examinations. This failure to encyst in the molluscan host provides an additional difference between *C. szidati* and the larva of *L. viviparae*.

Sewell (1922) divided the Vivax group of furcocercariae into two subgroups, and subsequent investigators (Faust, 1922; Szidat, 1933) have proposed additional subgroups on the basis of excretory patterns and tail structure. Cable (1938) redefined and simplified the classification to some extent, accepting three of the subgroups previously proposed, viz., the Vivax, Tetis, and Tauiana Subgroups. In defining the Vivax subgroup, he overlooked the fact that the cercaria of *L. viviparae* lacks furcal fin-folds and a recognizable ventral sucker. By possessing three pairs of flame cells in the tail-stem, and lacking a ventral sucker and furcal fin-folds, both *C. szidati* and the larva of *L. viviparae* overlap the Vivax and Tetis subgroups. It therefore becomes necessary to combine these subgroups and recognize at the most, only two subgroups of Vivax furcocercariae: (1) the Vivax subgroup which includes those with flame cells in the tail-stem and (2) the Tauiana subgroup whose members lack such flame cells. Indeed, further studies on life histories may demonstrate that even this distinction is of minor importance, and may warrant further simplification, even to the extent of combining the Vivax groups with other types of holostome cercariae. This would be warranted if further studies should reveal that the complex nature of the ascending excretory tubules in Vivax cercariae represents merely a precocious development of the so-called "reserve system" characteristic of the strigeids.

SUMMARY

Cercaria szidati sp. nov. is reported from *Campeloma sp.* collected from the Tippecanoe River, Indiana, U.S.A. The species is described and compared with other furcocercariae of the Vivax group and the classification of the group is discussed.

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EXPLANATION OF PLATE, p. 268

All figures pertain to *Cercaria szidati*

ABBREVIATIONS

ce	cercarial embryo	in	intestine
cg	cephalic glands	le	lateral excretory tubule
ct	caudal excretory tubule	mb	muscle band
ev	excretory vesicle	me	median excretory tubule
ft	furcal excretory tubule	os	oral sucker
hf	primordium of holdfast organ	ph	pharynx
ic	Island of Cort		

FIG. 1. Cercaria, ventral view.
FIG. 2. Sporocyst.

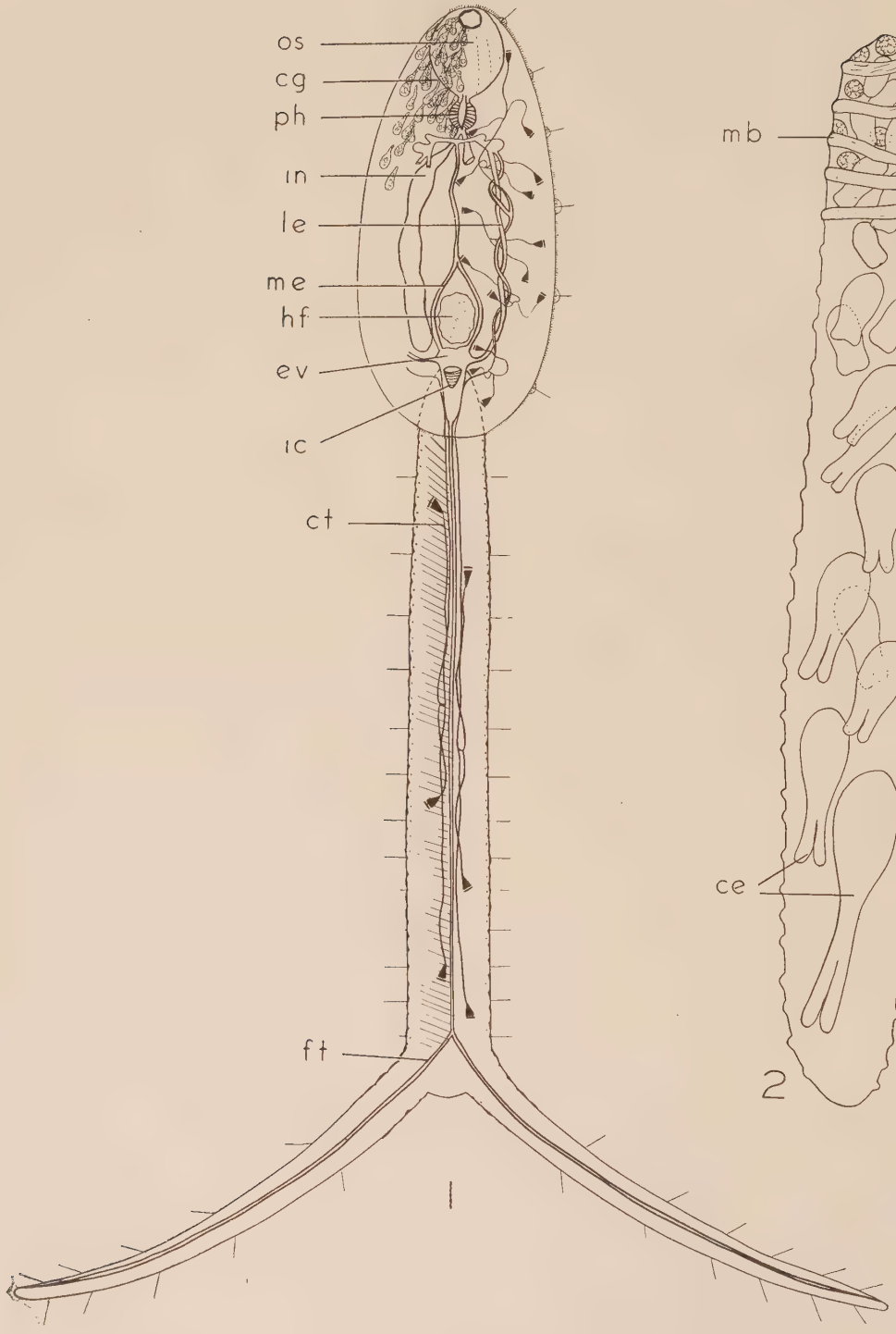

PLATE I

TWO NEW NEMATODES FROM THE AQUATIC BEETLE *HYDROUS TRIANGULARIS* (SAY)[1]

A. C. Todd

Louisiana State University

The worms described below were recovered from specimens of the large water scavenger beetle, *Hydrous triangularis* (Say), taken in Nebraska and Louisiana. The new nematodes belong to the family *Thelastomatidae* Travassos, 1929 and to the subfamily *Thelastomatinae* Travassos, 1920. They have been placed in the genus *Pseudonymus* Diesing, 1857 and perhaps constitute the first record of the genus from the United States.

Pseudonymus brachycercus n. sp.

(Measurements in millimeters)
Figs. 1–3

With the characters of the genus as given by Diesing (1857).

Male: Unknown.

Female: 3.15–4.11 long by 0.218–0.295 wide. Mouth opening prismoidal and surrounded by a circumoral elevation which bears eight papillae submedially arranged. Lateral organs or amphids present. Second annule enlarged, 0.015–0.025 long, forming a distinctive cephalic ring. Esophagus 0.43–0.50 long, consisting of an anterior cylindrical portion 0.328–0.385 long by 0.0375–0.0475 (at nerve ring) wide, followed by a short isthmus 0.0125–0.02 long and terminating in a valvular bulb 0.0825–0.103 long by 0.095–0.113 wide. Nerve ring 0.233–0.295 from anterior end of esophagus. Excretory pore elevated, posterior to base of esophagus, 0.52–0.65 from anterior end of body. Anterior end of intestine somewhat dilated; anus 0.361–0.438 from posterior end of body. Tail bluntly attenuated. Reproductive system amphidelphic. Vulva 1.772–2.616 from anterior end of body; lips not salient. Ovejector directed somewhat laterally; uteri bifurcating near middle of body. Eggs oval, 0.078–0.088 long by 0.048–0.055 wide, ensheathed in a spiral filament and containing a fully formed embryo when deposited.

Host: Hydrous triangularis (Say).

Location: Large intestine.

Distribution: U.S.A. (Nebraska, Louisiana).

Holotype: Female, U.S.N.M. Helm. Coll., 36896; *Paratype:* Female, No. 36896.

Pseudonymus leptocercus n. sp.

(Measurements in millimeters)
Figs. 4–6

Male: Unknown.

Female: 2.47–2.94 long by 0.145–0.22 wide. Mouth opening prismoidal and surrounded by a circumoral elevation which bears eight papillae submedially arranged. Lateral organs or amphids present. Second annule enlarged, 0.015–0.017 in length, forming a cephalic ring. Esophagus 0.385–0.423 long, consisting of an anterior cylindrical portion 0.293–0.33 long by 0.035–0.037 (at nerve ring) wide, followed by a short isthmus 0.017–0.02 long and terminating in a valvular bulb 0.065–0.08 long by 0.075–0.085 wide. Nerve ring 0.2–0.25 from anterior end of esophagus. Excretory pore elevated, posterior to base of esophagus, 0.484–0.546 from anterior end of body. Anterior portion of intestine somewhat dilated; anus 0.381–0.463 from posterior end of body. Tail filiform. Reproductive system amphidelphic. Vulva 1.450–1.710 from anterior end of body; lips not salient. Ovejector directed laterally; uteri bifurcating near middle of body. Eggs oval, 0.078–0.088 long by 0.048–0.055 wide, ensheathed in a spiral filament and containing a fully formed embryo when deposited.

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[1] Contribution No. 65 from the Dept. of Zoology, Entomology and Physiology, Louisiana State University.

Host: Hydrous triangularis (Say).
Location: Large intestine.
Distribution: U.S.A. (Nebraska, Louisiana).
Holotype: Female, U.S.N.M. Helm. Coll., No. 36897; *Paratype:* Female, No. 36897.

DISCUSSION

The genus *Pseudonymus* was erected by Diesing (1857) to receive *Oxyuris spirotheca* which had been described by Györy in 1856 from the aquatic beetle, *Hydrophilus piceus;* the worm then became *Pseudonymus spirotheca* (Györy, 1856) Diesing, 1857. Galeb (1878) described and figured four species of nematodes from hydrophilid beetles, among which was *Oxyuris* (*Helicothrix*) *spirotheca* from the beetle *Hydrophilus piceus;* he erected the subgenus *Helicothrix* to accommodate the four worms. Stiles and Hassall (1905) indicate *Helicothrix* to be a synonym of *Pseudonymus* and designate *P. spirotheca* as the type of its genus.

Basir (1941) establishd the genus *Galebiella* for two worms, *G. galebiella* and *G. istamabadi,* from aquatic beetles in India. He distinguished his genus *Galebiella* from the genus *Leidynemella* Chitwood, 1934 but did not refer to the genus *Pseudonymus.* The genus *Galebiella* agrees with the genus *Pseudonymus* noticeably in that both possess enlarged cephalic annules and in other morphological characters; they both are parasitic in aquatic beetles. *G. istamabadi* appears closely related to *P. spirotheca* which has been designated as a type species as mentioned above. From Basir's descriptions and figures *Galebiella* would appear to differ from *Pseudonymus* in that its eggs are not embryonated when deposited and are not ensheathed in a spiral filament. The tails of *G. galebiella* and *G. istamabadi* are conical and Galeb indicated that the tail of *P. spirotheca* was similar, among others, to that of *Hammerschmidtiella diesingi* (Hammerschmidt, 1838) Chitwood, 1932 and *Leidynema appendiculatum* (Leidy, 1850) Chitwood, 1932 which do not have conical tails.

The genus *Zonothrix* Todd, 1942 was established by the present writer for *Z. tropisterna* a parasite of a water scavenger beetle, *Tropisternus nimbatus. Oxyuris* (*Helicothrix*) *hydroi* Galeb, 1878 was renamed *Zonothrix hydroi* (Galeb, 1878) Todd, 1942. The genus *Zonothrix* was separated from its closely related genus, *Pseudonymus,* by the absence of cephalic rings, the location of the vulva at the posterior third of the body and the prolonged condition of the anterior lip of the vulva.

Of the two new worms, *P. brachycercus* appears to resemble *Pseudonymus hydrophili* (Galeb, 1878). The length of the female *P. hydrophili* is given by Galeb as 4 mm which is within the limits of the length of *P. brachycercus,* i.e., 3.15–4.11 mm. The two worms cannot be further distinguished because a full description of *P. hydrophili* was not given. *P. leptocercus* can be distinguished from *P. brachycercus* by its smaller size (2.47–2.94 mm compared to 3.15–4.11 mm) and because it has a filiform tail.

SUMMARY

1. The genus *Pseudonymus* Diesing, 1857 has been recorded from water scavenger beetles in the United States.

2. Two new parasitic nematodes, *Pseudonymus brachycercus* and *P. leptocercus* have been described from the water scavenger beetle, *Hydrous triangularis* (Say), in Nebraska and Louisiana.

3. The genus *Galebiella* Basir, 1941 has been related to the genus *Pseudonymus* Diesing, 1857.

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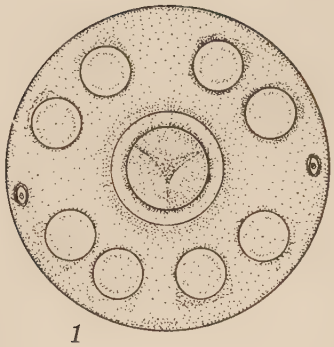
Explanation of Plate, p. 272

Fig. 1. *Pseudonymus brachycercus.* Head of female, en face view.
Fig. 2. *P. brachycercus.* Adult female.
Fig. 3. *P. brachycercus.* Egg.
Fig. 4. *P. leptocercus.* Head of female, en face view.
Fig. 5. *P. leptocercus.* Adult female.
Fig. 6. *P. leptocercus.* Egg.

All drawings were made with the aid of a camera lucida.



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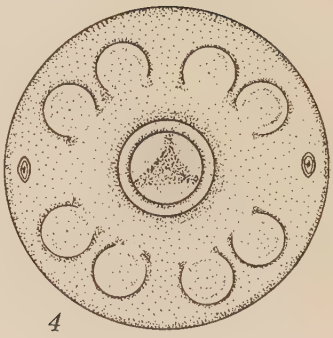

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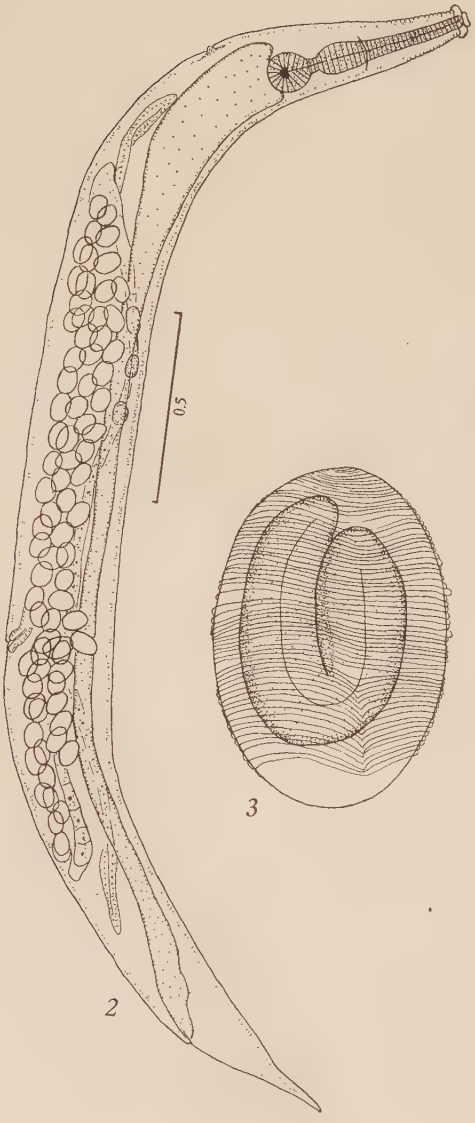

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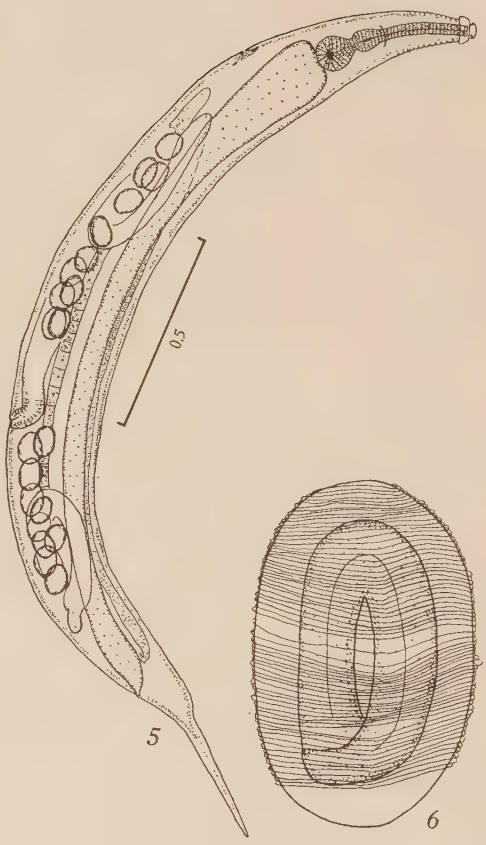

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PLATE I

RESEARCH NOTES

A NEW SPECIES OF *MESOCESTOIDES, M. KIRBYI,* FROM *CANIS LATRANS*

Several lots of *Mesocestoides* collected from *Canis latrans* in California, and sent to the writer for identification, differ from species of this genus hitherto described in several respects, and are therefore considered a new species, for which the name *Mesocestoides kirbyi* is proposed.

Diagnosis: Length of strobila 19 to 30 cm, maximum width 1.7 to 2 mm. Scolex 350 to 470 μ wide and 360 to 440 μ long. Suckers 230 to 300 μ long and 130 to 150 μ wide, with slit-like openings, 170 to 200 μ long. Calcareous corpuscles few and scattered. Mature segments nearly as long as wide when relaxed. Gravid segments strikingly bell-shaped, 2.2 to 4 mm long and 1.1 to 1.5 mm wide, junction with anterior segment only 200 to 500 μ wide. Testes 100 to 120 in number, 45 to 65 μ in diameter. Cirrus pouch 180 to 210 μ long and 130 to 150 μ wide. Ovaries

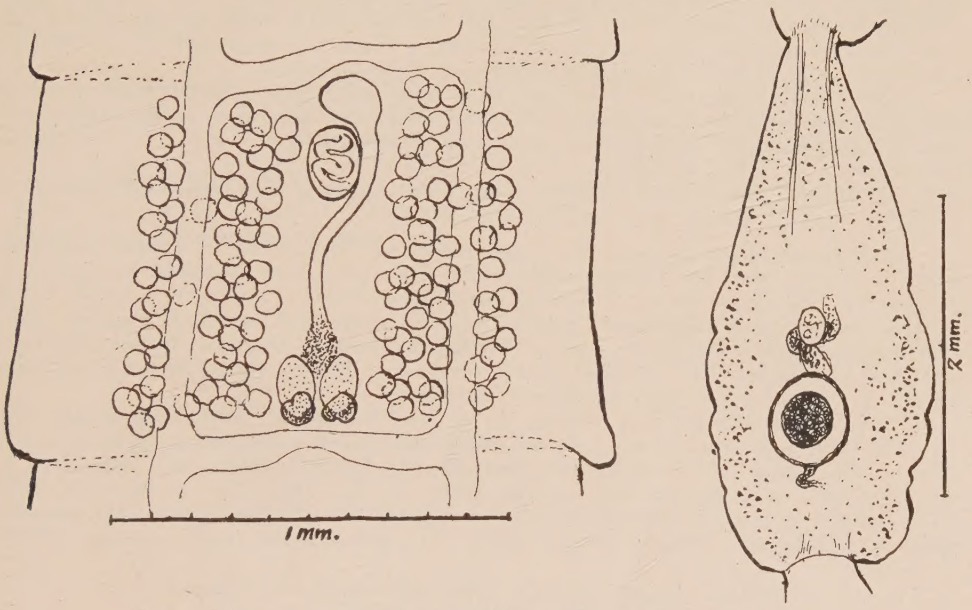

FIG. 1. Mature and gravid proglottids of *Mesocestoides kirbyi,* n. sp.

elongate, 120 to 170 μ long and 65 to 85 μ wide, almost contiguous. Vitelline glands ventral and slightly posterior to ovaries, 50 to 70 μ in diameter. Uterus curves around cirrus pouch, ending in club-shaped enlargement anterior to it. Egg sac forms as in *M. latus,* by thickening of walls of posterior part of uterus, differentiating into carrot-shaped organ with thick fibrous walls, posterior portion of which forms the capsule. Capsule 450 to 560 μ in diameter; egg mass 280 to 320 μ in diameter.

Host: Canis latrans.

Location: Intestine.

Locality: Hastings Natural History Reservation, California.

Type: U. S. Nat. Mus. Helm. Coll. No. 36898.

This species differs from all previously described forms in the large number of the testes, and from all but *M. lineatus* in the large size of the cirrus sac. It differs also from all except *M. manteri* in the bell-shape of the gravid proglottids. Except for the large number of testes this species has a strong resemblance to *M. lineatus.*—ASA C. CHANDLER, *Rice Institute, Houston, Texas.*

TWO ERRONEOUS RECORDS IN AMERICAN LITERATURE OF THE CAUSATIVE AGENTS OF MYIASIS

Because of our relatively scant knowledge of the immature stages of Diptera, determinations of larvae are often erroneous, and some misidentifications are perpetuated through repeated publication. In the case of myiasis-producing species, another source of error is failure to interpret the clinical data correctly, infestations of the vaginal and anal regions, or contamination

of stools, being often misinterpreted as gastrointestinal myiasis. However, when records that are evidently based on accurate observation are accompanied by reasonably good photographs or drawings, they leave something tangible for future workers to consider.

Blankmeyer (1907, J. Am. Med. Assn. **48**: 1505) reported a severe case of intestinal myiasis caused by *Fannia canicularis* (L) (reported as *Anthomyia canalicularis*). Later (1914, J. Am. Med. Assn. **63**: 321), for some unstated reason, he decided that his previous determination was wrong and that the larvae in question were *Anthomyia pluvialis* (L.). But for a European record, which needs reexamination, this is the only record of this latter species as a producer of myiasis in man that the writer has been able to find. The drawing in Blankmeyer's first paper indicates clearly, however, that he was dealing with a species of *Fannia,* possibly *F. scalaris* (F.); it was not *F. canicularis* and most certainly not *Anthomyia pluvialis,* which is of an entirely different type.

Felt (1928, N. Y. State Mus. Bul. **274**: 145–176) recorded the removal of several larvae of *Wohlfahrtia vigil* (Walker) from a cyst in the eye of a man in New York State. The record was unusual, since *W. vigil* is known to attack only subcutaneous tissues in very young animals, including the human species. It had not previously been reported in ocular myiasis, or in adults. The photograph accompanying Felt's report, however, is clearly that of the larva of *Oestrus ovis,* which has frequently been recorded as producing conjunctivitis in man.

Both these erroneous records have been cited by subsequent workers and textbooks of medical entomology.—MAURICE T. JAMES, *Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture.*

PHLEBOTOMUS LIMAI FONSECA IN THE UNITED STATES (DIPTERA: PSYCHODIDAE)

In the United States there are five known species of *Phlebotomus: P. vexator* Coquillett, which is widespread in the southern states, having been taken from Maryland to California; *P. diabolicus* Hall, from Texas; *P. texanus* Dampf, from Texas; *P. stewarti,* a species which is being described from California by Mangabeira and Galindo; and the fifth is a species which I first collected from a hollow tree at Annie Pond, near Florence, Alabama. Dampf (1938, An. Fac. Nac. Cien. Biol. 1: 119) mentions another species from Florida, but we do not as yet know whether it is one of those already described or a new one.

The specimens from northern Alabama are so close to *P. limai* Fonseca that, in spite of a few differences in the morphology, I do not believe they can be considered to represent a new species. The female of *P. limai* has been redescribed by Coutinho (1940, Rev. Mus. Paul. 1: 333), and the male by Barretto and Coutinho (1940, An. Fac. Med. Univ. S. Paulo **16**: 127). Table 1 gives a comparison of the measurements recorded for *limai* by these authors with measurements of 4 males and 5 females from Alabama. The palps are practically identical. In the

TABLE 1.—*Comparison of* P. limai *from Brazil and Alabama*
(Measurements in microns)

	Females		Males	
	Brazil	Alabama	Brazil	Alabama
Palp				
I		47– 52	24– 32	31– 36
II	170–189	161–177	121–135	125–135
III	148–183	161–177	108–121	125–135
IV	72– 86	77– 88	54– 67	68– 77
V	175–217	203–229	162–216	198–244
Wing				
α	629–757	560–620	483–583	460–530
β	221–314	300–360	232–244	270–340
γ	221–349	210–270	151–209	200–230
δ	189–279	140–250	116–233	120–170
α/β	2.0–3.1	1.7–2.0	2.0–2.5	1.4–2.0
Spermatheca				
Length	70	60– 80	.....	.....

wing one can find the only real difference between the two forms; in the Alabama specimens α is shorter and β is longer, so that α/β is somewhat larger in the flies from Brazil. The male terminalia are identical. The only difference in the spermatheca seems to be a longitudinal seam that may occur in some of the North American specimens. Mr. R. M. Damasceno, who examined several specimens which I had given to Dr. P. C. A. Antunes, sent me several drawings which emphasize the differences mentioned above, but a study of the larger amount of material I have

indicates that these differences are not clear-cut. Dr. Ayroza Galvao agres with me that the two are closely enough related to be considered as belonging to the same species.

The presence of *P. limai* in the United States presents an interesting problem in distribution. So far it has been reported only from several localities in the State of Sao Paulo, Brazil (Fonseca, 1935, 12th Cong. Int. Zool.: 1497; Coutinho, 1940; Barretto and Coutinho. 1940) where it does not seem to be particularly abundant (Galvao and Coutinho, 1940, Rev. Ent. **2**: 427). In addition to the flies captured in the hollow tree at Annie Pond in Alabama, I have identified specimens in the collection of the U. S. National Museum from Clinton, Mississippi, and Rockingham, North Carolina, as well as specimens in the Chicago Natural History Museum from Rockingham, North Carolina, and Selma, Alabama. Some of the females had been taken while biting. It would seem that the species has a wide distribution in the southern United States and one might expect to find it in Central America and northern South America, or perhaps in Florida and southward through the West Indies. I have tentatively identified as *limai* several specimens that Dr. Pablo Anduze sent to me from Venezuela.—L. E. Rozeboom, *Department of Parasitology, Johns Hopkins School of Hygiene and Public Health, and the Health and Safety Department, Tennessee Valley Authority.*

BARROELLA N. NOM. FOR *KIRBYELLA* ZELIFF, 1930, HOMONYM

Zeliff (1930, Am. J. Hyg. **11**: 740–742) described a flagellate from the termite, *Kalotermes* (*Calcaritermes*) *brevicollis*, and proposed a new genus, *Kirbyella*, to contain it. The name *Kirbyella* had been given previously (Kirkaldy, 1906, Entomologist **39**: 248) to a genus of insects and was not available. Accordingly, a new genus *Barroella* is erected to contain the flagellate species and the new combination *Barroella zeteki* is formed to replace *Kirbyella zeteki*.—C. Courson Zeliff, Pennsylvania State College.

CANCELLATION OF PROGRAM

The program of the American Society of Parasitologists, scheduled for Cleveland, Ohio, September 11 and 12, 1944, was cancelled except for the annual business meeting of the Society and the Symposium which was arranged jointly with the American Society of Zoologists, the American Society of Tropical Medicine and Section N (Medical Sciences) of the American Association for the Advancement of Science.

Titles and abstracts of papers submitted for the Cleveland meeting, together with a list of officers and new members, are published in the Supplement to the August number of the Journal of Parasitology.

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